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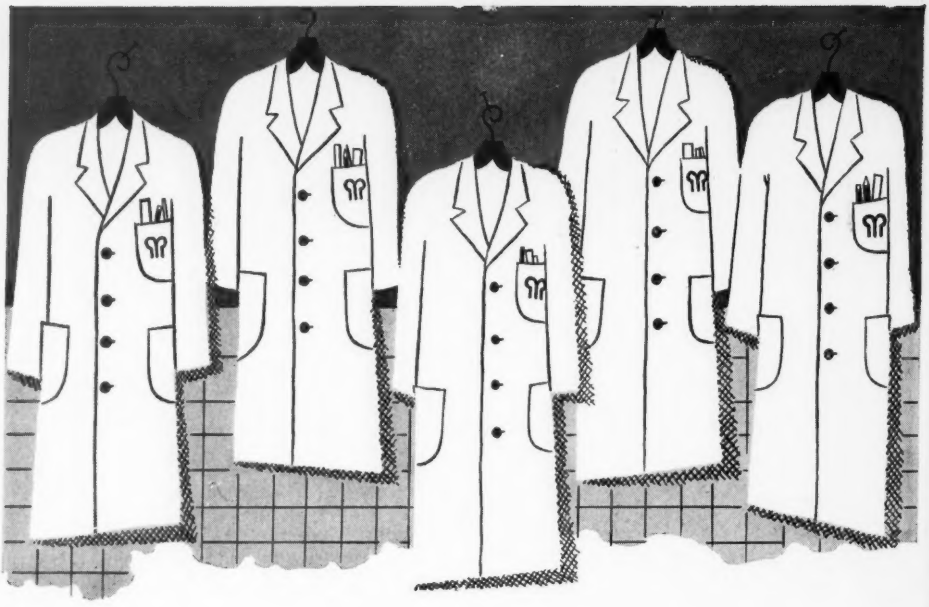

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ON THE PREPARATION OF HUMAN INSULIN FOR EXPERIMENTAL USE*

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THE purpose of this paper is to describe our experience in the preparation of human insulin for experimental use. The method is a modification of one described by Somogyi, Doisy, and Shaffer (1) for the preparation of beef insulin.

Others have presented evidence (2) indicating that insulins from various sources, including human, are immunologically identical. Studies in this laboratory have not been in accord with this conclusion, in that a marked difference was observed between the response to human insulin and commercial beef and pork insulins in a patient with insulin-resistant diabetes (3) and also in a rabbit immunized with commercial insulin (4).

METHOD

Fifty pancreases were collected at the autopsy tables of the Boston City Hospital, Beth Israel Hospital, and Massachusetts Memorial Hospitals from patients dying with heart disease, liver disease, cerebral accidents, malignancy, renal disease, and various other causes. Cases of diabetes, tuberculosis, or severe sepsis were excluded. The age of the patients varied from 12 to 90 years, the average age being 60 years. An effort was made to obtain material as soon after death as possible without disturbing the routine of the respective departments of pathology. The elapsed time from

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death to autopsy ranged from two to twenty-seven hours, with an average of 10.7 hours.

The pancreas was trimmed of excess fat by the pathologist, and a small block of tissue removed for routine microscopic study. The remainder was weighed and taken immediately to the laboratory.

The pancreatic tissue (40–200 Gm. per patient; average 82 Gm.) was ground in a household meat grinder in the refrigerator and mixed with 1.5 ml. of extracting fluid per gram. The extracting fluid consisted of 35 ml. of 1:4 dilution of concentrated H_2SO_4 per liter of 95 per cent ethanol. The pooled ground tissue was stored for several weeks in a large glass jar until 10 to 20 pancreases had been collected. The quantities given in the following section are based on 1 kilogram of pancreas.

The acid alcohol extract was separated from the grindings by filtering through gauze and pressing out as much liquid as possible. The extraction was then repeated with 1000 ml. of 70 per cent alcohol. After standing for two hours at room temperature, the solid matter was again removed by filtering through gauze. The filtrates were then combined and passed through filter paper. The final filtrate (about 2500 ml.) was transferred to a large dish, and the alcohol was evaporated by directing the stream of an electric fan over the surface. As the alcohol evaporated, the lipids precipitated out, and the aqueous layer became less colored. When the odor of alcohol was gone, at which time the volume was reduced to about $\frac{1}{4}$, the liquid fats were removed from the amber-colored aqueous layer in a separatory funnel, and the solids were removed by filtration.

Sodium chloride to a concentration of 25 per cent was added to the filtrate in a beaker, whereupon a dark green precipitate settled out. On standing for a few days in the ice box, more precipitate formed and adhered to the beaker, facilitating decantation. The supernatant fluid was poured off through a Büchner funnel, care being taken not to disturb the precipitate until most of the supernatant liquid had been poured off, in order to avoid clogging the filter paper.

Following the addition of 100 ml. of dilute HCl, most of the precipitate dissolved, forming a turbid green-brown solution containing some insoluble material. The suspension was transferred to a centrifuge tube, and 15 Gm. of NaCl was added, whereupon a precipitate reappeared. After standing overnight in the ice box, the tube was spun at 2000 rpm for 30 minutes and then decanted through a Buchner filter in order to collect a portion of the precipitate which floated. The precipitate was then dissolved in 25 ml. of dilute HCl, again producing a turbid green-brown solution, the pH of which was 1.85. Preparatory to filtration through a Seitz filter the pH was adjusted to 7.8 using N/10 NaOH. As the iso-electric point of insulin was passed, a heavy dark green precipitate formed and redissolved. The solution was filtered through a Buchner filter (under suction) and then through a sterile Seitz bacterial filter, through which buffered saline of pH 7.4 was first passed until no change in the pH of the saline solution occurred. Adjustment of the insulin solution to pH 7.8 and rinsing of the filter pad with buffer solution were done in order to avoid possible shift towards the iso-electric point of insulin (pH 5.35) with consequent precipitation and loss in the filter pad. The fine dark green precipitates, collected by filtration through the Buchner and Seitz filters, were discarded. Ten per cent phenol was added to make a final concentration of 0.5 per cent, and the pH was lowered to 2.5 with sulfuric acid. The solution was again hazy at this point, but cleared upon centrifugation at 1800 rpm for 30 minutes. The sediment, which was

dark green in color, was discarded, leaving a clear, amber-colored supernatant liquid, which was decanted and placed in sterile vials.

Assay

The insulin was first assayed by its ability to produce convulsions in white mice (5). Small numbers (5 to 10 mice) were used for purposes of orientation, and then the human insulin was compared with that of commercial insulin (Lilly U80) in larger groups (46 mice for each preparation). The assay of the final preparation was 21–25 u per ml. by this method. A second assay was done in rabbits using the "cross over" method of Marks (6), which is based on the per cent reduction in blood sugar following the subcutaneous injection of insulin. Blood sugars were determined by the micromethod described by Nelson (7), using the Folin method of protein precipitation (8). Five rabbits were used, each being tested with human insulin and commercial insulin (0.75 units/Kg.). By this method the preparation contained 24 u/ml., the estimated error being approximately 10 per cent for the number of animals used.

RESULTS

A total yield of 2500 units (106 ml. of a solution containing 24 units per ml.) was obtained from 50 pancreases. This represented an average yield of 50 units per pancreas or 0.62 units per gram. The final product was a clear, amber-colored liquid with a slightly greenish hue. It contained 0.34 mg. of nitrogen per ml., only 47 per cent of which could be ascribed to the insulin present. When 6.4 units (0.1 u/Kg.) were given intravenously to a 140-pound human subject with apparently normal carbohydrate metabolism, a normal insulin tolerance curve was obtained. The fasting blood sugar was 71 mg./100 ml.; 15 minutes after the injection it was 39 mg./100 ml.; at 30 minutes, 29 mg./100 ml.; at 45 minutes, 42 mg./100 ml.; at 1 hour, 60 mg./100 ml.; at 1½ hours, 70 mg./100 ml.; and at 2 hours, 105 mg./100 ml.

DISCUSSION

The purpose of this study was to obtain a quantity of human insulin for an experimental study of insulin resistance. Assays on two individual pancreases were made in order to determine what effect the interval between death and processing of the pancreas might have on the yield of insulin. According to rough assays in mice, a pancreas obtained twenty-seven hours after death contained one-quarter as much insulin as one obtained three hours after death.

In studies on the insulin content of the human pancreas from diabetic and nondiabetic individuals, Scott and Fisher (9) found that the nondiabetic pancreas contained an average of 173 units or 1.7 units per gram,

whereas the pancreas from diabetic individuals contained an average of less than 40 units per pancreas or 0.4 units per gram. They extracted only sufficient amounts for assay by a method which may be more efficient than ours, but which would be cumbersome and expensive for producing insulin in any quantity. There are several factors which may account for the lower yield obtained by us (50 units per pancreas or 0.62 u/Gm.) as compared to that obtained by Scott and Fisher from their nondiabetic group (173 units per pancreas or 1.7 u/Gm.):

1. They obtained pancreases from persons who died suddenly; we obtained ours from patients dying in the hospital, generally after a considerable period of debility.

2. The average age in their group was 41.2 years; in ours it was 60.4 years.

3. Their material was slightly fresher than ours, being obtained on an average of 9.6 hours after death as against 10.7 hours. This slight difference probably did not play a significant part.

4. The pancreases obtained by them were larger than those obtained by us, weighing an average of 119.7 grams compared with 91.6 grams. Part of the difference (about 15 grams) can be ascribed to tissue removed for microscopic study by the pathologist. Some difference in weight may be due to variation in the thoroughness with which fat and adventitious tissue was trimmed from the pancreas. It is very likely, however, that there was a real difference in the average weight of the organs in the two series of approximately 25 grams. This may be due to factors 1 and 2 mentioned above. Weight differences alone can effect only the yield per pancreas, and not the yield per gram.

5. Difference in method of extraction may very well account for the difference in yield. In our process, for example, considerable insulin activity may have been lost by allowing the pancreatic hash to remain for long periods of time in the extracting fluid.

A rough estimate of the relative efficiency of the two methods was attempted. A single pancreas was ground, and the grindings were divided into approximately equal portions which were then weighed. One portion was extracted by Scott's method. The other portion was extracted by the method described in this paper. All manipulations were carried out as in the preparation of the lot of insulin described earlier, including the elapse of one month, during which the hash stood in the refrigerator after addition of extracting fluid. Seitz filtration was also carried out. The numerous manipulations required by the method we used and the small size of the sample in this instance would probably decrease the yield, if it affected it at all, because the insulin lost on filters and glassware would be proportionately large. Assays were carried out in mice, using 80 to 120 mice for each prepara-

tion. Scott and Fisher's method yielded 1.6 u/gram of pancreas; our method 1.1 u/gram, or 65 per cent as much. This would indicate that the low yield obtained by us from 50 pancreases, a yield which was only 35 per cent of that obtained by Scott and Fisher, cannot be attributed entirely to the difference in the method used for extraction.

It is possible that a good part of the difference in the yield of insulin per pancreas between our group and that reported by Scott and Fisher (9) was due to the debility of the subjects from whom our pancreases were obtained. If this is true, then the striking difference noted by these observers between the insulin content of pancreases from diabetic and nondiabetic individuals was due only in part to diabetes per se, because in their series the nondiabetic subjects had died suddenly, whereas those with diabetes had died of associated debilitating disease.

SUMMARY

Human insulin suitable for injection into patients was prepared by a method requiring no special apparatus. The yield of insulin was approximately 50 units per pancreas or 0.62 u/gram.

Evidence is presented indicating that the insulin content of the pancreas is reduced in nondiabetic persons dying of debilitating disease.

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SYMPATHICOTROPIC (LEYDIG) CELL TUMOR OF THE OVARY WITH VIRILISM

REPORT OF A CASE

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MASCULINIZATION due to ovarian tumor is rare, and it is only within the past few years that clinical and laboratory data concerning such cases have been gathered with sufficient completeness to establish some of the diagnostic criteria. Some examples of the condition must still go unrecognized, or pass as states of "functional" amenorrhea or mild hirsutism. In this paper we report a case, cured by surgical removal of the tumor, which presents features of diagnostic interest, clinically and pathologically.

CASE HISTORY

Mrs. D. M., age 46, was admitted to the Montreal Neurological Institute on September 25, 1946. She complained of amenorrhea of eleven years' duration; increased hair growth on the body for six years; loss of libido, nervousness, increased fatigability and a tendency to baldness of the scalp for five years; and huskiness of the voice for one and a half years.

She had been in perfect health and had normal menstrual periods until eleven years before admission. At that time her periods suddenly stopped and she had no bleeding or discharge thereafter. Then an increased growth of hair appeared on her face. Three or four years later hair began to appear on her forearms and legs, and on the chest and abdomen. During the two years prior to admission hair appeared on her back and upper arms and on the back of her fingers, and everywhere it became thicker and coarser. Meanwhile the scalp hair became thin and bald spots appeared. For two years she had noticed that her muscles stood out like those of a man, and that she was much stronger, even though she found she tired quickly. The veins on her arms and legs became larger and more prominent and the skin became leathery. There was a gradual loss of 20 pounds in weight.

About a year and a half before admission her voice began to change, acquiring a masculine, husky quality, and the neck seemed to thicken. Her appetite for food increased so that she might get ravenously hungry, but she was more quickly satisfied than formerly. For a few months there had been mild numbness of the hands and legs. For ten years the patient took regular and nerve-racking electrolysis treatment for removal of facial hair.

In the past five years there had been complete lack of sexual appetite. There was increasing nervousness and selfconsciousness, and a contant indefinable sense of tension

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and jitteriness. She was very ill at ease and anxious when in the hospital ward with other women, and asked to be placed in a room by herself.

The family history was noncontributory and there was no history of other endocrine disorder. The patient had had one pregnancy resulting in a boy now 18 years old and alive and well. No subsequent pregnancies had occurred although children were desired.

Physical examination: The patient was a nervous, apprehensive woman of medium height and build. The body contour was masculine, the muscles of the upper and lower limbs stood out strongly like those of a man, and were very powerful. The voice was low but not entirely masculine. Hair over the scalp was thinned and there were several bald patches. Hair over the rest of the body was dark, thick and wiry. It grew especially

TABLE 1. GLUCOSE TOLERANCE CURVE—CAPILLARY BLOOD

	1946	1947
	mg. %	mg. %
Fasting	93	67
1 hour	190	124
2 hours	159	122
3 hours	102	—
4 hours	88	89
5 hours	106	96

TABLE 2. HEMATOLOGY

	1946	1947
RBC	5,500,000	4,300,000
WBC	10,000	8,500
Hb	110%	84%
Color index	1.0	0.98

on the chest, limbs and face. The breasts had lost their normal fullness and the hips were relatively narrow.

There was no evidence of kyphosis or scoliosis of the spine, and there were no striae cerulae. There was no marked evidence of acne. The skin was of normal color and there was no lividity or suffusion. The optic fundi and visual acuity were normal, and the visual fields were full. The remainder of the examination, save for a generalized hyperreflexia, showed nothing abnormal. The blood pressure, measured on many occasions, averaged about 130/84, and the blood Wassermann reaction was negative.

Special examinations: Stereoscopic anteroposterior and right lateral films of the skull showed no evidence of an intrasellar tumor, nor was there any hyperostosis cranialis interna. Stereoscopic films of the lumbar spine showed minimal osteoarthritic spurring, but there there was no evidence of intervertebral disc disease nor of osteoporosis. X-ray examination showed a normal chest. Cystoscopy, carried out by Dr. Magnus Seng, and contrast pyelograms failed to show any evidence of adrenal tumor.

Gynecologic examination by Dr. G. Simpson revealed moderate hypertrophy of the clitoris. The remaining organs were small but did not seem atrophic. No evidence of any large ovarian tumor could be made out.

The report of the vaginal smear, from the Gyne-cytology Laboratory (Dr. J. E. Ayre) was as follows: "The cytological picture is most unusual. Both vaginal and cervical smears show a complete absence of cornification (endogenous estrogen). The cells are all of an atypical, basal, atrophic type which we have seldom seen. Some of these show multinucleation with foamy appearance throughout the cytoplasm.

"Interpretation: 1. Total absence of cytological evidence of endogenous estrogen. 2. We cannot account for cells of this type. The picture is not typical of that found when male hormone is administered. Whether some adrenal hormones enter this picture we do not know."

TABLE 3. BLOOD ELECTROLYTES

	Plasma		Serum		
	Chloride	N.P.N.	Sodium	Potassium	Calcium
1946	mEq./L	mg. %	mEq./L	mEq./L	mg. %
Oct. 23	—	28.4	—	—	10.5
Oct. 24	101	20.0	142	—	—
Oct. 24	- - - - - - - - - - -		Operation	- - - - - - - - - - -	
Oct. 25	—	—	146	6.3	—
Oct. 28	97	27.5	142	5.0	—
1947					
Oct. 6	—	—	142	4.2	11.2
Oct. 7	101	23.0	142	4.35	11.0

TABLE 4. URINARY OUTPUT OF 17-KETOSTEROIDS, GLYCOGENIC CORTICOIDS AND CREATININE

	17-Ketosteroids	Corticoids	Creatinine
	mg./24 hrs.	units/24 hrs.	Gm./24 hrs.
1946			
Sept. 25 & 26	22.4	—	1.6
Oct. 2 & 3	22.8	63	1.7
Oct. 9 & 10	16.0	74	1.5
Oct. 24	- - - - - - - - -	Operation	- - - - - - - - -
Oct. 31 & Nov. 1	10.6	88	—
Nov. 3 & 4	12.9	—	—
1947			
Oct. 7 & 9	10.5	—	1.1

Blood examinations: A glucose tolerance curve (capillary blood) was normal (Table 1). A complete hemogram was normal save for moderate erythrocytosis along with slight leucocytosis, due to an increase in all forms (Table 2). Examination of the electrolytes of the blood showed no abnormality (Table 3).

Hormone excretion: The twenty-four hour output of 17-ketosteroids in the urine was moderately increased, as shown in Table 4. Urinary glycogenic corticoids were within normal limits.

A provisional diagnosis was virilism due either to adrenal cortical hyperfunction, or to ovarian tumor. Since both adrenals and both ovaries had to be explored, the Surgeon-in-Chief, Dr. Gavin Miller, elected to use a single abdominal incision. This was done in order to avoid the three incisions which would have been necessary for exploration of the adrenals and pelvic organs separately.

Operation: October 24, 1946. Surgeon: Dr. Gavin Miller.

The abdomen was opened under spinal anesthesia by a midline incision from the xiphoid almost to the symphysis.

The right adrenal gland presented no visible or palpable abnormality. The left adrenal appeared to be considerably enlarged. The right ovary measured approximately

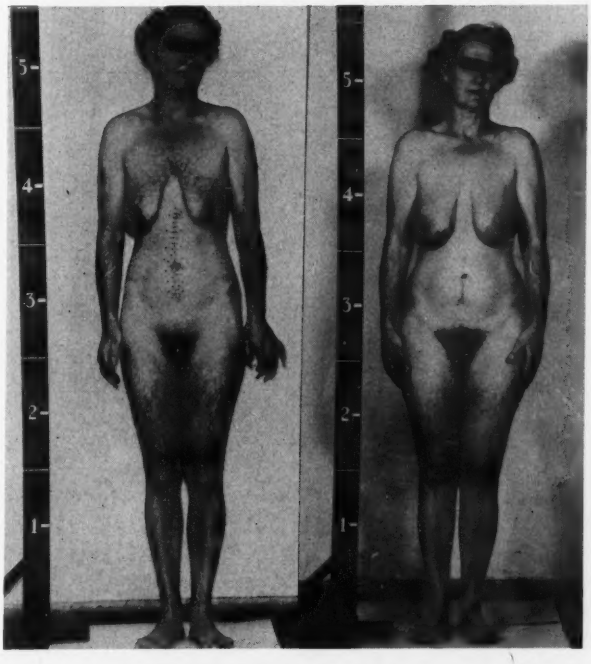

FIG. 1. Appearance of patient immediately after operation (left) and one year later (right). Note change in body contour and loss of excessive hair.

1.5 cm. in diameter and was fibrotic and calcified. The left ovary measured approximately 3.5 cm.; one pole was of meaty consistency, and appeared hyperemic. No other intra-abdominal abnormality was detected. It was not entirely clear whether a lesion was present in the left adrenal or left ovary. The left adrenal was dissected free and removed *in toto*. The left ovary and the fimbriated end of the fallopian tube were also removed. The abdomen was closed in layers without drainage, using steel wire.

The patient's postoperative condition was satisfactory. Five per cent glucose and plasma were administered during operation. Following operation, adrenal cortical extract was given, 10 cc. intramuscularly every four hours for three days, and the patient was kept on a low potassium diet. She sat up on the side of the bed one day after opera-

tion, sat in a chair on the second day and was walking on the fourth postoperative day.

Follow-up: No clinical change was noted in the two weeks of observation in the hospital after operation. There was, however, an almost immediate, though modest, drop in the excretion of 17-ketosteroids in the urine, as indicated in Table 4.

The patient was re-admitted in October 1947, one year after operation. She then stated that she had first noted a mental change beginning three weeks after operation. She became progressively more relaxed and lost the indefinable feeling of tenseness. It is of interest to note that at this time no change in her physical appearance was yet

FIG. 2. Cut surface of the ovary and tumor. Bulging, soft dark tumor tissue occupies the center, surrounded by narrow, pale capsule of ovarian tissue.

manifest, to make her feel less selfconscious. In two months the face began to clear of hair and the voice lost its huskiness. Normal menstrual periods were established in three months, after an absence of almost twelve years, and continued regularly thereafter. The skin became softer and smoother and hair decreased over the body and became finer. She gained 20 pounds in weight, mainly on the hips, thighs and breasts. The hair on her head became more glossy. The striking change in appearance is shown in Figure 1. Libido remained low but sexual satisfaction was normal. Pelvic examination showed some remaining hypertrophy of the clitoris and moderate enlargment of the uterus.

Gyne-cytologic examination of vaginal smears now showed normal evidence of endogenous estrogen with glandular and basal cells and some red blood cells and leuco-

cytes. The blood sugar curve was normal (Table 1). The moderate erythrocytosis and leucocytosis had disappeared (Table 3). The output of 17-ketosteroids in the urine remained normal (Table 4).

PATHOLOGIC EXAMINATION

The removed ovary measured 3.7 × 3 × 3 cm. and weighed 10 Gm. (Fig. 2). Its external surface was smooth, glistening and pale bluish grey in color. It had a soft rubbery consistency. Sections through its longest axis showed yellow-white ovarian tissue forming a capsule 2–4 mm. in thickness about a central tumor mass. The capsule of ovarian

FIG. 3. Microscopic appearance of the tumor. Ovarian stroma containing thick-walled blood vessels occupies the upper left half of the picture and the neoplasm the lower right. Note absence of any fibrous capsule. Hematoxylin and eosin stain. ×119.

tissue appeared incomplete at the ovarian hilus. No cysts were grossly visible in the ovarian tissue. The cut surface of the tumor bulged irregularly and consisted of soft, rather friable red-brown tissue having a coarsely lobular architecture. Irregular hemorrhagic zones, up to 0.7 cm. in diameter, were present in the central portion. At the periphery some of the tumor tissue showed indistinct yellow areas.

The left adrenal was unaltered in shape. When stripped of fatty tissue, it weighed 8.5 Gm. and measured 7 × 3 × 1.5 cm. The cortex was deep yellow and the medulla red-brown, and neither was considered grossly abnormal.

Microscopic examination: Blocks were taken from various regions of both specimens. These were fixed in neutral formalin, and sections were stained with hematoxylin and eosin and Masson's trichrome; stains for reticulum and fat were also made.

The capsule of the ovarian tumor was found to consist of ovarian stroma in which corpora albicantia but no follicles were seen. No fibrous capsule (Fig. 3) separated the

tumor tissue from the ovarian tissue. At the hilus of the ovary, the capsule of ovarian tissue was lacking and the tumor cells were bordered by the connective tissue of the mesovarium. The tumor was composed of polyhedral cells, arranged in strings, columns or in apparent haphazard manner. At no point in the tumor could any suggestion of tubular adenomatous structure be observed. A loose network of fibrous tissue accompanied the vessels which penetrated the tumor obliquely from the periphery. Thin spokes of fibrous tissue radiated from the vessels, disappearing between the cells of the neoplasm. The tumor cells (Fig. 4) had round or finely indented nuclei of lymphocyte size and occupied

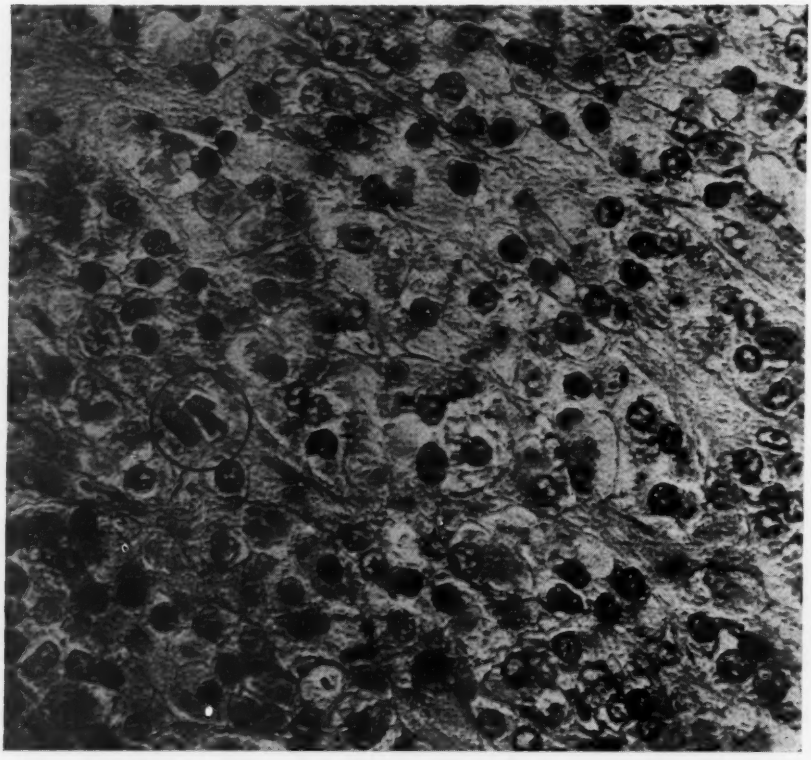

FIG. 4. A cell containing three characteristic crystalloids has been circled. Masson's trichrome stain. ×640.

about half the cell diameter. Nuclear membranes were distinct and the chromatin tended to be concentrated at the nuclear periphery. Most nuclei had a distinct, rather large, basophilic nucleolus. The cytoplasm was acidophilic, finely granular, and cell borders were usually sharp. In many of the cells there was a condensation of homogeneous cytoplasm about the nucleus, while at the cell periphery the structure was looser and paler. Mitoses were rare. No pigment was noted in the cytoplasm and vacuolation was not a feature. Many of the cells, especially near the periphery of the tumor, contained a bar-

shaped body with oblique ends, staining an amber color in Masson's trichrome preparation. This structure extended to the longest diameter of the cell and in some instances appeared to push the nucleus to one side, compressing it into an oval shape. Usually these crystalloids were surrounded by a narrow clear zone. Occasional cells were seen containing two or three of these bodies, lying roughly parallel to one another. In sections stained with haematoxylin and eosin the crystalloids were acidophilic, and appeared to merge with the cytoplasm of the cells containing them, so as to render their identification extremely difficult. Reticulum stains showed a delicate reticulum extending between the cells of the tumor. Frozen sections, stained with Scharlach R., revealed fine lipoid droplets in some of the cells; the majority, however, were fat-free.

Sections of the adrenal showed both cortex and medulla relatively broad and well preserved. The architecture and cellular detail of both regions were considered normal.

On the basis of the morphology of the tumor, it was designated as a tumor of the sympathicotropic cells of the ovarian hilus. There was no evidence that the tumor was malignant.

COMMENT

Clinical findings: Pituitary basophilism had to be excluded in this case. This was done on the basis that certain features were lacking; *viz.*, striae cerulae, osteoporosis, painful adiposity, vascular hypertension and increased glycogenic corticoids. The moderate increase is blood elements was interpreted as a normal male pattern rather than a true polycythemia, and it reverted to a normal female picture after operation. The remaining features, amenorrhea, increased hair growth, clitoral hypertrophy and other signs of masculinization pointed to either adrenal cortical hyperfunction or to a virilizing ovarian neoplasm. We also feel that the emotional aberrations were part of the endocrine picture.

This case also illustrates that a virilizing ovarian tumor is not necessarily associated with a greatly increased excretion of 17-ketosteroids in the urine. The average normal 24-hour output for a woman of this age is 11.0 mg. as determined at our clinic (1). Our patient excreted about 20.4 mg. per day before operation, and a normal amount thereafter. Very high outputs have been described hitherto only in cases of adrenal cortical tumors or hyperfunction. However, a normal output does not rule out either adrenal or ovarian tumor.

In a case such as this, when precise localization of the tumor cannot be arrived at on clinical findings, it is necessary to consider exploration by means of a large abdominal incision, in order that both adrenals and ovaries may be investigated at the one time.

Pathologic findings: In 1923 Berger (2) drew attention to certain cell groups in the hilus of the ovary, which, because of their intimate association with sympathetic nerve fibres of the region, he named sympathicotropic cells. The cells occur in the hilus of the ovary and in the mesovarium, being particularly frequent in the rete ovarii, and in the median

part of the mesovarium. They occur singly or in small collections, between the nerve fibers, or closely applied to the perineurium. They are usually in the immediate vicinity of a small blood vessel. There is considerable variation in size and shape from cell to cell. The cells usually are polyhedral in outline, and show a tendency to be arranged in short columns or small alveoli. They have a granular cytoplasm, which may show condensation about the nucleus, whereas the periphery is more pale staining and loose. Cell borders are fairly distinct. Within the cytoplasm small globules of fat may be found, and there is often a finely granular brown pigment. The most distinctive, though inconstant cytoplasmic inclusions are relatively large bar-shaped crystalloids with oblique ends. These occur singly or in groups of two or three within a single cell. The crystalloid usually fills the length of the cell, and may be of sufficient width to push the nucleus to one side. As a rule, it is surrounded by a clear zone of cytoplasm. Morphologically and microchemically these crystalloids are indistinguishable from the albuminoid crystalloids of Reinke, found in the interstitial cells of the testis.

These cell collections had been previously observed by von Winiwarter in 1910 (3) who held the opinion that they were pheochrome elements. Berger has been the chief proponent of the view that these cells are morphologically identical with the interstitial (Leydig) cells of the testis and has suggested that they may be the source of male hormone, which can be extracted from the ovary (4).

The cells reach their fullest development in the adult premenopausal ovary, but small groups of cells which are probably of identical nature, have been found in the ovaries of infants (5), and their senile remnants have been demonstrated in postmenopausal ovaries (2).

We believe that the tumor in this case exhibits morphologic features which identify it as a neoplasm of sympathicotropic cells (Leydig cells). Sympathicotropic cells are most numerous in the region of the ovarian hilus. The interruption of ovarian encapsulation of this tumor in the hilus is in keeping with origin from sympathicotropic cells in this region, with subsequent extension into the ovarian medulla. The cell detail and arrangement, together with the presence of the characteristic crystalloids, are typical of sympathicotropic cells, and argue against the possibility of origin from theca cells, lutein cells or adrenal rest tissue. The removed adrenal exhibits no abnormal features which warrant its consideration as the cause of virilization.

The remarkable clinical remission following the removal of the ovary indicates an endocrine influence ascribable, we believe, to the sympathicotropic cell tumor within it. This is in keeping with the suggestion by Berger (4) that the sympathicotropic cells of the ovary are a) morphologically identical with the interstitial cells of the testis, and b) may be the source of male hormone which can be extracted from the ovary.

It seems to us advisable to separate this ovarian tumor from the rather heterogeneous group of "arrhenoblastomas," and to apply to it the designation indicating its cells of origin. In doing so we have adhered to the name originally applied by Berger (2). It would perhaps be justifiable to refer to such tumors as Leydig cell tumors, or interstitial cell tumors of the ovary, names, which may be more indicative of the true nature of the cells. However, until a sufficient number of cases have been recorded for accurate analysis, it seems to be advisable to retain the designation of sympathicotropic cell tumor, which appears to have chronologic priority over other terms.

It is interesting to note that Selye (6), in discussing "arrhenoblastomas" states: "It is quite possible that the degree of Leydig cell development within the tubular adenomas could account for the virilization seen in some cases, but this has not yet been proven." The present case, in which no tubular structure was observable, appears to be in line with such a supposition.

Sympathicotropic cell tumors with virilization have been reported previously by Berger (4) and Dreyfus and Barrozo do Amaral (7). Berger's case was a woman of 50 years, who had symptoms of facial hypertrichosis, male pubic escutcheon, deep, hoarse voice, atrophy of the breasts, and marked enlargement of the clitoris, but who had a normal menstrual cycle. The symptoms had been present for eighteen years, but showed significant regression after removal of her uterus and ovaries for uterine fibromata.

Berger expresses the opinion (8) that the tumors described by Cosacesco *et al.* (9) and by Giordano and Haymond (10), whose sections he has examined, are derived from the same cells.

In discussions of masculinizing ovarian tumors, neoplasms of sympathicotropic cells are rarely mentioned.

Since so few cases of this rare neoplasm have been reported, it is felt that the addition of the present case may help to establish it as an entity.

SUMMARY

Clinical and pathologic features of a case in which there was a tumor of the ovarian sympathicotropic cells are described. Attention is drawn to the fact that this rare neoplasm is frequently overlooked in consideration of virilizing ovarian tumors.

Acknowledgment

The authors are indebted to the late Dr. Louis Berger, who kindly examined our material and suggested the diagnosis. Thanks are also due to Professor Theodore R. Waugh and Professor G. Lyman Duff for assistance in preparation of the material.

Addendum

Since this article was submitted for publication, a report of two similar cases has been published by William H. Sternberg (*Am. J. Path.* 25: 493 (May) 1949).

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AN ENDOCRINE FINDING APPARENTLY CHARACTERISTIC OF GOUT:

VERY LOW URINARY 17-KETOSTEROID EXCRETION WITH CLINICALLY NORMAL ANDROGENIC FUNCTION*

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WE HAD speculated on the role of androgens (Table 1) and other hormones in gout but deferred study of the problem because most gout patients are free of obvious endocrine disease. One of our gout patients, a vigorous alert 59-year-old tavern keeper, who complained of impotence and hair loss on the forearms, was then found to have a urinary 17-ketosteroid excretion of only 0.7 mg./day. In current endocrine theory, this finding implies either failure of both gonadal and adrenal androgen production, or a more obscure disturbance in steroid production or metabolism. This observation led to the survey of a group of gout patients reported here. Unusually low values for 17-ketosteroid excretion were found in all patients studied. An attempt will be made to explain this finding by detailed consideration of the various known causes for very low 17-ketosteroid outputs.

Material and Methods

The data were chiefly obtained from study of the 11 patients listed in Table 2. Data from other patients seen during the past few years have been quoted in the discussion. Certain other investigators, whose aid is acknowledged elsewhere in the text, have provided important related data.

The urate values are for total urates, determined colorimetrically. Normal values do not exceed 6.2 mg. per cent (1). 17-Ketosteroids were estimated by the procedure of Talbot, Butler and MacLachlan (2).

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TABLE 1. OBSERVATIONS WHICH SUGGEST A POSSIBLE RELATIONSHIP OF SEX HORMONES TO GOUT AND TO URATE METABOLISM
(The observations on which these statements are based are reviewed in detail elsewhere (3, 4, 5, 6).)

1. Most reported series of gout patients consist from 75 to 95 per cent of men.
2. Eunuchs are reputed to be free from gout.
3. In women, gout generally does not have its onset until shortly before or at the menopause.
4. In premenopausal gouty women, attacks of gouty arthritis frequently are reported to begin at the menses.
5. The average plasma urate level of normal adult men is higher than that of normal adult women.
6. The average plasma urate of gouty men is higher than that of gouty women.
7. In normal children, the average plasma urate level is lower even than that of normal adult women. The sex differential in plasma urate appears at puberty and is not present in normal children.
8. Men who inherit asymptomatic hyperuricemia do not generally show elevated urate levels until after puberty.
9. Women who inherit asymptomatic hyperuricemia do not generally show elevated urate levels until, or shortly before, the menopause.

Methods for determining 17-ketosteroids which do not include the separation of the neutral ketonic fraction with Girard T reagent may give results higher than those reported here. The Girard T separation eliminates nonketonic chromogens which are apparently disproportionately high in the urine of gouty patients.

RESULTS

17-ketosteroid excretion in gout: Table 2 shows the greatly diminished output of 17-ketosteroids observed in all 11 gout patients. The average of 3.24 mg./per day for the 10 men in this table is about one-third of the minimum normal value. We know of no other reported study of ketosteroid excretion in gout,[1] but Dr. William Wolf has told one of us (W.Q.W.) of observing moderately reduced 17-ketosteroid excretion in this disease.

The 5 male patients whose age was over 45 years had a mean daily 17-ketosteroid excretion of 2.79 mg. The 5 younger gouty males had a mean output of 3.64 mg. The known decline in ketosteroids with age (9) presumably accounts for this small difference.

[1] Urinary androgens were estimated biologically, but 17-ketosteroids were not determined in the patient with male pseudohermaphroditism(?) and typical gout reported by Rosenberg in 1942(7). Such patients are ordinarily found to have 17-ketosteroid outputs and urinary androgen excretory levels considerably above normal. The urinary androgen activity in this patient was equivalent to 2–8 mg. of androsterone per day. This is within normal limits for normal adult males, but distinctly low for patients of this type (8).

Abnormally decreased ketosteroid excretion occurred both during acute gouty arthritis and in asymptomatic interval gout. It would appear that neither the activity of the disease nor the severity of the constitutional reaction are responsible for the low ketosteroid output.

17-ketosteroid excretion in non-gouty hyperuricemia: We attempted to determine if the diminished 17-ketosteroid output was a direct consequence of hyperuricemia. Table 2 shows that 3 of 4 relatively healthy subjects

TABLE 2. BASIC DATA ON GOUT PATIENTS STUDIED FOR HORMONAL STATUS AND ON PATIENTS WITH NON-GOUTY HYPERURICEMIA

Patient	Sex	Age, years	Height, inches	Weight, pounds	Average serum urate, mg. %	B.M.R. %	17-ketosteroids, mg./24 hrs.*	Glomerular filtration rate, cc. per minute per 1.73 sq. M.	Hepatic function tests**
Chronic gouty arthritis; remission									
M.J.	F	48	59	133	13.3	+12	0.67	54	Normal
O.T.†	M	59	66	168	12.1	− 5	0.96	72	Normal
Interval gout									
S.K.	M	49	71	165	7.7	+ 3	0.69	90	Normal
W.S.	M	51	66	160	9.4	—	3.60	75	Normal
H.N.	M	49	54	144	7.6	—	5.90	86	Normal
M.P.	M	43	69	132	7.7	+ 5	6.60	74	Slight impairment
E.W.	M	23	70	178	6.6	—	1.74	116	Normal
A.S.	M	43	72	190	8.4	—	2.25	—	Normal
Acute gouty arthritis									
M.K.	M	37	70	270	10.5	−9‡	2.24	—	Normal‡
R.H.	M	44	68	200	7.2	—	5.35	—	Normal‡
B.V.	M	54	68	180	10.2	—	3.02	—	Normal‡
Average, all gout patients		*45*	*68*	*175*	*9.2*	1	*3.00*	*81*	
Non-gouty hyperuricemia									
TGB.♯	M	36	71	246	7.5	+ 8	4.3	—	Normal
KWI.♯	M	39	68	290	7.1	—	12.0	—	Normal
WOL.♯	M	30	73	215	6.4	− 5	10.0	121	Normal
SCH.§	M	39	67	145	6.3	—	18.5	107	Normal

* Normal values for urinary 24-hour 17-ketosteroid excretion:
Females: 7.0 to 12.0 mg. per 24 hours.
Males: 9.0 to 25.0 mg. per 24 hours.

** The group of tests used included: serum bilirubin, serum total protein and protein fractionation, serum cholesterol and cholesterol partition, thymo, turbidity, thymol flocculation and cephalin-cholesterol flocculation. In some cases, colloidal gold flocculation, alkaline phosphatase and bromsulfalein clearance were also determined. *Normal* indicates no abnormal result on any test.

† Complaints of impotence and hair loss on forearms; azoospermia found. Good response of both complaints to administration of testosterone propionate, 25 mg. parenterally, three times weekly, in about 6 weeks.

‡ These determinations were carried out during the interval stage.

♯ Diagnosis: possible hypothalamic(?) hyperuricemia. This diagnosis is applied to a group of 7 patients in whom unexplained hyperuricemia has accompanied signs and symptoms suggestive of hypothalamic disorder. The symptomatology has included: obesity (7 patients), R.B.C. over 5.0 million (5 patients), hypersomnia (5 patients). Additional findings in individual patients have been: hypercholesterolemia (326 mg. %), electrolyte changes resembling those of Cushing's syndrome, intermittent hypochloremia, familial pitressin-resistant diabetes insipidus with normal renal function tests, unexplained eosinophilia and lymphocytosis, habitual oliguria, and syndactyly. A case resembling KWI has recently been published by Mussio Fournier and Proto (10).

§ Cystinuria, familial.

with non-gouty hyperuricema excreted normal amounts of ketosteroid. The size of the group and the degree of elevation of plasma urate are not optimal because of the relative infrequency of suitable subjects. The results do suggest that 17-ketosteroid excretion is not necessarily diminished in non-gouty hyperuricemia.

TABLE 3. IN MALES WITH RHEUMATOID ARTHRITIS OR SPONDYLITIS, AVERAGE DAILY 17-KETOSTEROID EXCRETION IS NOT SO LOW AS IN GOUT

(Data on patients with rheumatoid arthritis and with spondylitis are unpublished results made available by Dr. A. P. Forbes and Dr. W. S. Clark of the Massachusetts General Hospital, Boston. For the purposes of classification, the one male and the one female patient with chronic gouty arthritis in remission have been considered to be in the interval phase.)

	Males		Females	
	Number	17-ketosteroids mg./24 hrs.	Number	17-ketosteroids mg./24 hrs.
Normal Adult, average values				
Massachusetts General Hospital	—	12.5	—	8.2
Michael Reese Hospital	—	12.0	—	9.0
Rheumatoid Arthritis (Massachusetts General Hospital)				
Rheumatoid polyarthritis	4*	9.7	11	4.6
Rheumatoid spondylitis	3	10.5	1	5.2
All cases	7	10.0	12	4.7
Gout (Michael Reese Hospital)				
Interval gout	7	3.1	1	0.7
Acute gouty arthritis	3	3.5	—	—
All cases	10	3.2	1	0.7

* The 17-ketosteroid outputs in two men with minimal constitutional signs were 11.0 and 11.0 mg. per 24 hrs. respectively. The two remaining patients, with more severe constitutional signs, had average 17-ketosteroid outputs of 10.5 and 6.4 mg. per 24 hours.

17-ketosteroid excretion in rheumatoid arthritis: It appeared important to compare the ketosteroid excretion of gouty men with that of men with rheumatoid arthritis and spondylitis to see whether diminished 17-ketosteroid excretion was specific for gout. This was made possible when Dr. Anne P. Forbes and Dr. William S. Clark of the Massachusetts General Hospital very generously made their unpublished observations available.

Table 3 summarizes comparative findings in gout and rheumatoid ar-

TABLE 4. CONDITIONS ASSOCIATED WITH VERY LOW 17-KETOSTEROID EXCRETION

DECREASED PRODUCTION OF 17-KETOSTEROID PRECURSORS
Associated with decreased 11-oxysteroid production
1. Pituitary failure
2. Severe hypothyroidism and myxedema
3. Adrenal failure in females
4. Adrenal plus secondary gonad failure in males*
5. Severe hepatic dysfunction
Associated with increased 11-oxysteroid production
1. Cushing's syndrome; some cases**
2. Alarm reaction, "resistance stage"
ABNORMAL TYPE OF STEROID PRODUCTION OR METABOLISM
Normal precursor not converted to 17-ketosteroid
1. Severe hepatic dysfunction
?Androgen produced not 17-ketosteroid precursor?
1. Arrhenoblastoma, some cases
2. Some cases of hirsutism in females†

* Pure testicular failure is not included in this table, since it is not believed to result in 17-ketosteroid outputs below 50% of minimum normal values.

** Cushing's syndrome actually seldom leads to very low values. However Albright (13) has argued for a primary increase in oxysteroid and decrease in ketosteroid production, making Cushing's syndrome analogous to the "resistance stage." He explains the normal to moderate elevation in ketosteroid excretion of many patients either as compensatory or, in the case of tumors, as mixed types of syndromes.

† The status of pure hirsutism without virilization as an androgenic manifestation is not firmly established. Hypersensitivity of the end-organ might be responsible. Moreover, some types of hirsutism occur in Cushing's syndrome when no other suggestion of virilization is present.

thritis. The tendency of gouty men to have lower 17-ketosteroid outputs than men with rheumatoid spondylitis or polyarthritis is apparent. The values in rheumatoid arthritis are consistent with those reported by Davison, Koets and Kuzell (11), whose data even suggest that ketosteroid excretion is occasionally high in rheumatoid arthritis.[2]

[2] Perdersen-Bjergaard and Tønnesen (12) have recently reported results of urinary androgen assays by the capon comb method in various groups of male patients, including 17 with chronic rheumatoid arthritis. Normal values were found to be from 5 to 25 c.u./24 hrs. Men with prostatic hypertrophy and impotence had outputs within the normal range. The average androgen excretion in patients with rheumatoid arthritis was low, being approximately 5 c.u./day. It did not exceed 8 c.u./day in any of the 17 analyses.

In gout, 17-ketosteroid excretion is low even during the interval stage when the disease is apparently completely quiescent, except for hyperuricemia. Comparable male patients with rheumatoid arthritis of low activity have normal ketosteroid outputs. The presence of diminished 17-ketosteroid outputs in all stages of activity would appear to be peculiar to gout.

Other causes of very low 17-ketosteroid outputs: Table 4 summarizes relevant conditions which regularly produce 17-ketosteroid outputs below about 2.5 mg./day, and groups these causes according to the responsible underlying disturbance in function. In females virtually all androgen originates in the adrenal cortex and decreased ketosteroid excretion implies decreased adrenal androgen production or a metabolic abnormality. In males, androgen is produced by both testis and adrenal. Ketosteroid outputs below 2.5 mg./day, in the absence of an anomaly of metabolism, result only from decreased androgen production by both these glands (14–17).

Five men and the one woman in our series had 17-ketosteroid outputs below 2.5 mg. per day. In at least these 6 patients, one may assume the cause to be either deficiency in both adrenal and gonadal androgen production, a qualitative abnormality in the pattern of steroids produced, or a disturbed intermediate steroid metabolism. Each of these possibilities will be considered.

Adrenal, pituitary and thyroid insufficiency: The average daily excretion of 17-ketosteroids is regularly below 2.5 mg./day in panhypopituitarism, in females with Addison's disease, in those males with Addison's disease in whom hypogonadism develops, and in severe hypothyroidism or myxedema (15, 16, 18- through 31).

Table 5 compares certain clinical and laboratory findings in adrenal, thyroid or pituitary insufficiency with those in interval gout. This table indicates that the diminished 17-ketosteroid excretion in gout cannot be referred to thyroid, pituitary, or adrenal failure.

Liver function: 17-ketosteroid excretion is decreased in severe chronic liver disease, probably as a result of a number of endocrine mechanisms (30, 32).

Table 2 summarizes the results of testing the 11 gout patients with a battery of sensitive liver function tests. With one minor exception, the results were entirely normal, a finding consistent with that obtained when we applied the same tests to a larger group of gout patients (33). In general, the results indicated that abnormalities in hepatic function were unusual in gout unless complicating disease were present.

Normal results with function tests did not eliminate the possibility that a specific enzymatic deficiency of the liver might have resulted in disturbed steroid metabolism and in low ketosteroid outputs. Fortunately a direct test of this possibility was available. Following intramuscular injection of

TABLE 5. SOME OBSERVATIONS WHICH INDICATE THAT THE LOW 17-KETOSTEROID OUTPUTS OF PATIENTS WITH GOUT CANNOT BE EXPLAINED AS THYROID, ADRENAL, OR PITUITARY FAILURE

(The statement concerning life expectancy in gout as compared with the non-gouty obese may be deduced by comparing Bauer and Klemperer's remarks on the prognosis of gout (34) with those of Evans on the prognosis of obesity (35). For a recent, but characteristic, statement on the accomplishments of the gouty, see McCracken, Owen and Pratt (36). The table deals with the findings in interval gout, because no complicating inflammatory lesion is present in these patients.)

Some signs and symptoms of thyroid, adrenal, or pituitary failure	Findings in interval gout patients
1. Anorexia	Obesity usual; average 20 pounds over weight (37)
2. Asthenia	Unusual vigour and accomplishment usually noted by reviewers (36)
3. Hypotension	Hypertension in about 50 per cent (37)
4. Fasting hypoglycemia	Never reported. Diabetes mellitus in about 2 to 6 per cent
5. Tendency to Addisonian crisis	Never reported
6. Decreased life expectancy	Life expectancy equal to that of equally obese non-gouty
7. Addisonian pigmentation	Never reported
8. Tendency to increased serum potassium, diminished sodium and chloride	Not present ((38), p. 96, table 1)
9. Decreased B.M.R.	B.M.R. is normal (Table 1)
10. Hypercholesterolemia (chiefly in hypothyroidism)	Serum cholesterol is usually normal in interval gout (33)
11. Anemia	Average values; R.B.C. 5.08 million, hematocrit 46, hemoglobin 15.7 Gm. per cent (37)
12. Lymphocytosis	Average values: 26.7 per cent of total leukocytes; 1924 per cu. mm. (37)
13. Tendency to eosinophilia	Average values: 2.6 per cent of total leukocytes; 181 per cu. mm. (37)*

* Slide differential

50 mg. of testosterone propionate, normal males excrete 14 to 70 per cent as extra urinary ketosteroid within seventy-two hours. Inability to convert injected testosterone to ketosteroid is one of the abnormalities responsible for low ketosteroid excretion in cirrhotics. In 4 patients with Laennec's cirrhosis, Lloyd and Williams (32) recovered only 0 to 7 per cent of the injected testosterone as ketosteroid in the seventy-two hours after administration.

TABLE 6. 17-KETOSTEROID EXCRETION FOLLOWING INJECTION OF 50 MG. OF TESTOSTERONE PROPIONATE SHOWED NO ABNORMALITY IN THE CONVERSION OF ANDROGEN TO 17-KETOSTEROID

(Normally, 14 to 70 per cent of the injected material is recovered as urinary 17-ketosteroid within 72 hours of the time of injection. The average increase in urinary 17-ketosteroids per day during this postinjection period is normally between about 3 and 12 mg./day when estimated on the pooled 72-hour specimen. Urine samples in the two experiments below were only 48-hour collections, but despite this, the recoveries were normal.)

	17-Ketosteroid excretion		Recovery
	Before testosterone mg. per day	After testosterone mg. per day	Per cent in 48 hrs.
Patient O.T.	1.0	10.0	36%
Patient S.K.	0.7	10.3	38%

Table 6 summarizes results obtained when 50 mg. of testosterone propionate was administered intramuscularly to 2 gouty men whose basal 17-ketosteroid excretions were 1.0 mg. per day or less. The normal recoveries of 36 per cent and 38 per cent in forty-eight hours indicate unimpaired ability to convert testosterone to ketosteroids.

Renal function: Renal function was sufficiently well preserved to render "retention" of 17-ketosteroids unlikely. Glomerular filtration rate averaged 81 cc./min./1.73 sq.M. (Table 2), which is somewhat higher than the average value of 64 cc./min./1.73 sq.M. obtained in 20 unselected gout patients (unpublished data). This value represents only moderate renal impairment and is accompanied neither by clinical signs and symptoms of renal insufficiency nor by biochemical evidence of renal impairment such as elevations in serum urea or inorganic phosphate. The results of the testosterone injections, noted above, also suggest that when ketosteroids actually were produced, they could be excreted without difficulty.

Biologic androgen activity: 17-ketosteroids are not the sole end-product of androgen metabolism; and, in addition, certain 17-ketosteroid precursors, such as etiocholanolone, are not potent androgens. Nevertheless, androgenic function and 17-ketosteroid excretion are moderately well correlated under most circumstances, and a 17-ketosteroid output of less than 2.5 mg. per day in a mature adult man will generally be associated with obvious evidence of deficient androgen activity.

There is little about gout as a disease (Table 1), or about the gouty as individuals, to indicate that their diminished ketosteroid excretion actually is associated with the expected hypogonadism. The only two reports of hypogonadism in gout are those of Lichtwitz (39) and of Hench (40), but neither case can be considered spontaneous hypogonadism. In the former, hypogonadism followed eight years of severe polycythemia vera and, in the latter, mumps orchitis during adolescence was apparently responsible.

Good evidence indicated that the patients had had normal gonadal function throughout most of adult life and some findings suggested that it remained normal when the low 17-ketosteroids were found. Nine of the 10 men were married. Six of the married men had children: 2 had one child each, 3 had two children each, and 1 had four children. Five of the 9 men over 30 exhibited definite advanced bitemporal baldness, while 1 showed a milder degree. Creatine and creatinine excretion were studied in 6 men (Table 7). The creatinine coefficient, an index of functional muscle mass, averaged 21.1 mg./Kg./day, well within the normal limits of 20 to 26. Significant creatinuria of above 1.0 mg./Kg./day was absent in 4 of the 6 patients. These observations are presumptive evidence for normal androgenic status rather than absolute proof. Further documentation in the form of sperm counts, FSH assays, androgen outputs and similar data is now being sought.

DISCUSSION

The data detailed in this article may be summarized briefly. In gout, the average daily outputs of urinary 17-ketosteroids are below half of the minimum normal values. This decrease appears not to depend to any important extent upon the stage of the disease, or upon the age of the patient. It is present in entirely asymptomatic patients with interval gout. Since it does not uniformly occur in non-gouty hyperuricemia, it may not be a necessary consequence of hyperuricemia. Low 17-ketosteroid outputs do not occur in men with mild rheumatoid arthritis. The decreased ketosteroid excretion is therefore a property of gout itself and not a general phenomenon of the arthritides.

Decreased 17-ketosteroid excretion in gout is not satisfactorily explained by any of the currently accepted mechanisms. Outputs below 2.5 mg. per

day in the male cannot be explained solely as testicular deficiency but must be interpreted as dual failure of adrenal and gonadal androgen production, or as resulting from an abnormal pattern of steroid secretion or metabolism (14–17). All low outputs in the female must be referred to deficient adrenal androgen production or to metabolic error. Six patients in this series were found to have a 17-ketosteroid excretion below 2.5 mg./day.

Pituitary insufficiency, Addison's disease in females, Addison's disease with secondary testicular failure in males, and severe hypothyroidism regularly produce comparable decreases in 17-ketosteroid output. There are, however, no findings in gout which suggest obvious adrenal, pituitary, or thyroid insufficiency. We also considered the possibility that hepatic impairment, either generalized or as a specific enzyme deficiency, might be responsible. A battery of sensitive liver function tests gave no evidence of diffuse hepatic impairment in these or other gout patients (33). Testosterone propionate, administered parenterally, was recovered as urinary ketosteroid in the usual amounts. Since testosterone is now believed to be the most important physiologic androgen (41), normal recoveries after injection appeared to eliminate an isolated hepatic enzyme defect which might prevent metabolism of testosterone to ketosteroid. Renal function was sufficiently well preserved to render unlikely the theoretical "retention" of 17-ketosteroids.

A remaining possibility is that the endocrine situation in gout corresponds to the "resistance stage " of Selye's general adaptation syndrome (42). The "resistance stage" may be roughly defined as the phase of convalescence from acute injury or the state of dynamic equilibrium during chronic injury or disease, in which the organism manifests increased tolerance to a repetition or to an increase in intensity of the original injurious stimulus. It is partially characterized by increased corticoid (glycocorticoid, 11-oxysteroid) production and excretion, and by a concomitant reduction in 17-ketosteroid production and excretion (20, 24, 26, 27, 28, 29, 31).

Patients who exhibit the characteristic steroid excretion pattern of the "resistance stage" are generally chronically ill or are convalescing from severe illness or operation. The gout patient, on the other hand, may exhibit his characteristic low 17-ketosteroid excretion during the interval phase when he is apparently entirely well, except for hyperuricemia. Good health alone does not prove the absence of "resistance stage" endocrine status in interval gout, since the gouty conceivably might exemplify good adjustment to chronic injury.

We have reported preliminary attempts to evaluate this problem indirectly by study of a number of metabolic variables known to reflect the rate of adrenocortical glycocorticoid production (37). This and other re-

ports have indicated that alterations in adrenocortical function may be important in the mechanism of acute gouty arthritis (33, 37, 43–47). However, there is no clear evidence to indicate that adrenocortical hyperfunction is continuously present in interval gout. One example may be cited. In spontaneous adrenocortical hyperfunction of the Cushing type, and in certain stress situations, differential leukocyte counts show marked eosinopenia and lymphopenia. Similar changes may be produced in man by the administration of the purified adrenocorticotropic hormone or by certain metabolic steroids of the adrenal cortex. Table 5 shows that eosinopenia and lymphopenia are not present in interval gout.

Robinson, Conn, Block and Louis (45) have recently presented convincing evidence of the absence of chronic "resistance stage" status in one carefully studied gout patient to whom adrenocorticotropin was administered. Before the hormone was administered, the patient, then in a prolonged interval phase, was found to have a normal output of 11-oxysteroids (measured as formaldehydogenic steroids). ACTH was found to elicit a normal increase in 17-ketosteroid excretion. Patients in the "resistance stage" show both an increase in basal 11-oxysteroid output and a substandard increase in 17-ketosteroid output under stress conditions, unlike this gout patient. We have recently confirmed these findings in 2 additional gout patients (48).

Elimination of the general adaptation syndrome exhausted the established causes of very low 17-ketosteroid output. It was then necessary to see whether the hypogonadism, expected to occur with low 17-ketosteroid outputs, actually did occur in gout. In only one of the patients studied was evidence of hypogonadism found. In the others biologic androgen activity appeared normal.

Table 7 summarizes observations on a larger group of gouty men. It will be noted that almost all gout patients marry, and that the number with children and the number of children per patient are typical of normal adults. Six of the 27 patients with children sired one or more after the onset of clinical gout, which occurred at an average age of 41 years. This is unusually late, and suggests that some patients forgot their initial attack. It may well be, therefore, that the number of patients siring children after the initial attack was actually considerably greater. Our 2 patients with six and seven children respectively both reported the onset of clinical gout to have preceded the birth of *all* children. Since normal gonadal function certainly existed when clinical gout was present in these patients, the findings imply that it may exist when decreased ketosteroid excretion is present.[3]

[3] One patient (H.N.) noted exacerbations of facial acne 24 to 72 hours before the onset of gout attacks. Similar exacerbations of acne may be produced by the administra-

TABLE 7. SOME BIOLOGIC INDICES OF ANDROGEN ACTIVITY IN GOUT PATIENTS

(Fertility data, particularly in regard to children born after the onset of clinical symptoms, are partly those assembled by Dr. C. W. Cotterman from unpublished data of Smyth, Cotterman, and Freyberg. The table includes male patients only; female patients will be discussed in a later communication.)

	Yes	*No*
Married?	32	5
If married, any children?	27	5
Any children born after onset of clinical gout?*	6	21
Bitemporal baldness?†	50	45
Significant creatinuria?‡	3	10

Creatinine coefficient

	Mg./Kg./day
Average, normal adult males	20.0–26.0
Average, 13 gout patients	20.7

Fertility of male gout patients

Number of children	Number of patients
0	5
1	10
2	11
3	0
4	4
5	0
6	1
7	1

Average number of children per patient: *1.9*

* The average age at the first reported gout attack was 41 years, an unusually late age. It would seem probable that some of the patients may not have recalled their very first attack, hence that the number of patients siring children after the first attack is underestimated in this table.

† Well established baldness in 33; thinning to mild in 17.

‡ Defined as an excretion of creatine in excess of 1.0 mg. per Kg. per day.

The clinical data suggest the low 17-ketosteroid excretions of the gouty to coexist with normal biologic androgen activity. There are a few excep-

tion of sufficient adrenocorticotropin (49) and are believed to be referable to the demonstrable release of preformed adrenal androgen by this hormone. Exacerbations of skin lesions as gouty prodromata were frequently commented upon in the older literature (39), and a form of "gouty eczema" was believed to occur. Talbott (36) found evidence of psoriasis or eczema in less than 10 per cent of his series. However, those patients who did have either of these conditions reported an exacerbation of local skin symptoms before or during an attack of acute gouty arthritis, in Talbott's experience.

tions (Table 2 and (39, 40)), but this is not surprising in a group of men who average 45 years of age when first seen by the physician. Detailed reasons for rejecting the established causes of diminished 17-ketosteroid outputs as possible explanations have been given. Previous investigators have described only two conditions, both in females, in which an abnormal increase in biologic androgen activity was not accompanied by a proportionate increase in 17-ketosteroid excretion. In arrhenoblastoma (50), 17-ketosteroid excretion may be normal or low when marked masculinization is present, and ketosteroid excretion with such tumors rarely is comparable to that seen in virilization due to adrenocortical hyperplasia or tumor. In simple hirsutism in women, 17-ketosteroids were subnormal in approximately one-third of the patients investigated by Bissell and Williams (51). Unfortunately, hirsutism as a solitary sign is not well established as purely androgenic in origin. There appear to be no reports of conditions in which normal androgenic activity accompanies low ketosteroid outputs.

The concurrence of very low 17-ketosteroid outputs with normal biologic androgen activity in gout appears to be a new endocrine finding. We believe the diminished ketosteroid output to be referable to decreased production of normal precursors because exogenous testosterone, the presumptive chief active normal gonadal hormone (39), was normally converted to ketosteroid. The finding of normal biologic androgen activity means that the gouty must produce some type of androgen. The *working hypothesis* adopted postulates that, in gout, biologic androgen activity is maintained by an androgen which does not make an important contribution to urinary 17-ketosteroids when metabolized. Since hyperuricemia may be inherited by either sex (3, 4, 5, 6, 30, 31, 34) and since clinical gout occurs in both men and women, such an abnormal androgen would presumably originate in the adrenal cortex. The finding of such a substance might well clarify the complex metabolic problems implicit in the observations listed in Table 1.

CONCLUSION

Decreased urinary 17-ketosteroid output has been found in all of a group of 11 gout patients. It was present in all stages of the disease, including asymptomatic interval gout. A similar decrease was not found in a small group of patients with idiopathic hyperuricemia, nor was it present in males with rheumatoid arthritis or spondylitis.

A review of the currently available endocrine explanations for decreased 17-ketosteroid output of the degree found indicated none to be acceptable. Injected testosterone was recovered as urinary 17-ketosteroid to the usual extent and no defects in hepatic function were found with a representative group of sensitive liver function tests. Renal function was well enough pre-

served to make "retention" of 17-ketosteroids improbable. In spite of accumulating evidence that altered adrenocortical function may be important in attacks of gout, there is no evidence of "resistance stage" endocrine status during interval gout.

Biologic evidence of androgen activity was normal in 9 of the 10 men in this group. A review of a much larger series of patients gave additional evidence that hypogonadism is not clinically prominent in patients with gout.

The finding of very low outputs of urinary 17-ketosteroids in the presence of normal biologic androgen activity appears to be a new endocrine finding which is characteristic for gout. Our working hypothesis assumes that, in gout, biologic androgen activity is maintained by an androgenic hormone which does not make an important contribution to urinary 17-ketosteroids when metabolized.

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It is a pleasure to thank Dr. Anne P. Forbes and Dr. William S. Clark of the Massachusetts General Hospital for permitting us to study and to quote their unpublished observations on 17-ketosteroid excretion in rheumatoid arthritis and rheumatoid spondylitis. We also wish to thank Dr. Charles W. Cotterman of the Heredity Clinic, University of Michigan, who reviewed the unpublished data of Smyth, Cotterman and Freyberg in order to help determine the percentage of gout patients having children after the onset of clinical symptoms. Dr. Walter Bauer very kindly reviewed the manuscript before it was submitted for publication.

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PREGNANCY IN ADDISON'S DISEASE

REPORT OF FOUR PATIENTS

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INTRODUCTION

THE occurrence of pregnancy in patients with Addison's disease presents certain features of physiologic interest and poses several problems in therapy. Most standard textbooks state that the maternal adrenals enlarge in pregnancy, though their contribution to the normal progress of gestation remains for the most part unknown. An approach to this problem has been made by Venning by determining urinary excretion of substances derived from the adrenal in 9 normal pregnant women (1). In the first trimester of pregnancy a temporary increase was observed in the excretion of glycogenic corticoids to more than double that found in normal nonpregnant women, but early in the second trimester, there was a return to the values found before pregnancy. A second and greater rise to 5 times these values was noted in the latter part of pregnancy, with finally a fall before delivery. The 17-ketosteroid excretion increased gradually throughout pregnancy when assayed with m-dinitrobenzene (Zimmermann reaction). This increase was interpreted by Venning as due to an increase in 20-ketosteroids rather than in 17-ketosteroids, since the latter as determined with antimony trichloride (Pincus method) remained unaltered.

Certainly the presence of the adrenal glands is not essential either for the establishment of pregnancy or for its maintenance. There are available 29 reports of 39 pregnancies in women with Addison's disease, 30 of which continued to term (2–29). Although in certain of these reports the data given are insufficient to establish the diagnosis of adrenal insufficiency, in the great majority the records are of unquestionable authenticity. In only one such patient has a study of the steroid excretion been reported (26). In this patient, Samuels and associates found that the excretion of estrogens, pregnanediol and of 17-ketosteroids (assayed by a modification of the Zimmermann reaction) during the last two trimesters corresponded to the amounts excreted in normal pregnancy. An assay of the corticoid excretion was not made.

Reports concerning gonadal function in adrenalectomized animals have been conflicting; however, it appears definite that estrous cycles continue

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in such animals if they are maintained in good condition by the ingestion of salt, and that pregnancy may occur (30, 31). It has also been claimed that pregnancy and pseudopregnancy may even prolong the survival time of untreated adrenalectomized animals (32, 33). In addition, postpartum lactation has been observed in adrenalectomized rats maintained in good condition with sodium chloride added to the diet (34).

Despite the fact the pregnancy may occur and progress to term in the absence of adrenal cortical function, it remains a serious complication for the patient with Addison's disease. By far the majority of authors have observed that the health of such patients deteriorates during gestation, and in 8 of the 12 well documented reports, death of the mother has resulted during the course of pregnancy, immediately post-partum or within a short period thereafter. From a consideration of the normal physiologic changes in pregnancy, difficulties peculiar to the patient with Addison's disease might be expected. The nausea and vomiting in the first trimester, with accompanying disturbance in electrolyte balance, might not be well tolerated. In the last trimester, on the other hand, the increased blood volume and the possible contribution of hormones from the fetal adrenals might have a favorable effect upon the adrenal insufficiency. However, serious difficulties might be anticipated at the time of delivery as a result of blood loss, and again in the postpartum period during the diuresis which follows the withdrawal of placental progesterone. Finally, in the patient whose adrenal disease is on a tuberculous basis, there is a possibility of activation of the tuberculosis during pregnancy.

It is to be expected that the problem of pregnancy in Addison's disease will become increasingly frequent, since the life expectancy and general well being of these patients has been substantially increased with recent advances in therapy. It seems worthwhile, therefore, to report the findings in 4 such patients observed at the Presbyterian Hospital.

METHODS

Four patients with documented Addison's disease were studied during 5 pregnancies. One pregnancy ended in a spontaneous abortion, a second was terminated by dilatation and curettage and a third by hysterectomy. In 2 of the patients the pregnancy went to term. One of these was delivered by Caesarian section, and the other had a spontaneous delivery.

Serum sodium concentration was calculated by summation of the chloride values and carbon dioxide content expressed as milliequivalents plus 10, or was determined directly by use of an improved type of internal standard flame photometer with an accuracy of ± 1 per cent (35): potassium was determined after precipitation as chloroplatinate (36) or directly with the flame photometer. Determinations of estrone plus estradiol in

24-hour urine specimens were made by the the method of Jailer (37); gonadotropins by the method of Levin and Tyndale (38); sodium pregnanediol glucuronidate[1] by the technique described by Venning (39); corticoid by the glycogen deposition test of Venning, Hoffman and Browne (40);[2] and 17-ketosteroids, for the most part, by the Pincus reaction (41), although a few values were obtained by the Zimmermann reaction (42).

CASE HISTORIES

Case 1, P. H. #627658, E. G. was a 32-year old white housewife who was admitted in the seventh week of her first pregnancy. The diagnosis of Addison's disease had been established seven years previously, at which time she had an eleven-month history of increasing pigmentation of the skin and a four-month history of nausea, vomiting, and weight loss of 10 pounds. There was no background of tuberculosis. On physical examination increased pigmentation was evident although it did not extend to the mucous membranes. Her blood pressure was 95/65. Laboratory studies at that time revealed the following values: serum sodium, 125 mEq. per liter; serum potassium, 5.5 mEq. per liter, fasting blood sugar, 80 mg. per cent; urinary excretion of 17-ketosteroids (Zimmermann reaction), 2.9 mg. in 24 hours; and basal metabolic rate, minus 17 per cent. A roentgenogram showed that the chest was normal and an abdominal film showed no evidence of adrenal calcification. A tuberculin test, 0.1 cc. of O.T. in a 1:100,000 dilution, gave negative results. With the administration of 3 mg. of desoxycorticosterone acetate (DCA) daily by subcutaneous injection she remained in fairly good health for the ensuing seven years, with the exception of one admission in a crisis precipitated by iritis. At this time the value for serum sodium was 110 mEq. per liter and for blood sugar, 45 mg. per cent.

At the time of admission for pregnancy, a dilatation and curettage was advised. Beginning the day before the contemplated procedure she received whole adrenal extract (ACE) intramuscularly in addition to the maintenance dose of DCA, 5 cc. the day before operation, 10 cc. on the day of operation and 10 cc. the day after. The ACE used was the hog adrenal lipo-cortex of Upjohn, 1 cc. of which is the equivalent of 4 mg. of corticosterone or of 2 mg. of Compound E. Scopolamine and demerol were used as preoperative medication, and an infusion of glucose and saline was started before moving the patient to the operating room. Cyclopropane was selected as the anesthetic agent. Dilatation and curettage was performed by Dr. D. A. D'Esopo, the operation lasting ten minutes and the anesthesia, twenty minutes. In spite of this short procedure with negligible blood loss, upon return to the ward, the patient went into a state of shock: the pulse became feeble, the blood pressure fell to 82/40 and temperature to 96°. Response to shock therapy and transfusion, however, was prompt and her subsequent course uneventful.

One year later this patient died at the Norwalk hospital following an upper respiratory infection. Dr. L. A. Giuliano has kindly informed us that an autopsy performed by Dr. R. N. Barnett showed extreme atrophy of the adrenals.

Case 2, P. H. #811755, M. S. was a 31-year old housewife admitted in the fourth month of her second pregnancy. This patient's first pregnancy has been reported by Sheldon (27). At that time Addison's disease was suspected from the characteristic pigmentation of the skin and mucous membranes and a blood pressure of 100/70, and the

[1] These determinations were very kindly made by Dr. L. Levin.

[2] We wish to thank Dr. E. H. Venning for performing the determinations.

diagnosis was established by salt withdrawal in a Cutler-Power-Wilder test. X-ray examination of the chest and of the adrenal area showed them to be normal at that time and also subsequently. A tuberculin test reaction was reported as negative, whereas in 1946, 0.1 cc. of O.T. in a 1:10,000 dilution gave a mildly positive reaction. There was no family history of tuberculosis. The postpartum course following her first pregnancy was reported as stormy, with high fever and blood pressure at shock level for seven days after delivery. Her general condition in the intervening years had deteriorated and with the onset of the second pregnancy there had been increasing weakness. An intercurrent respiratory infection led to admission to an outside hospital, where severe nausea and vomiting resulted in a weight loss of 6 kilograms and necessitated repeated infusions. Because of her poor condition and known previous difficulties following delivery, termination of this pregnancy was considered advisable.

Beginning the day before operation, she was given ACE intramuscularly in addition to her maintenance dose of DCA. The same preoperative medication and anesthesia were employed as in Case 1. Dr. H. C. Taylor performed a supravaginal hysterectomy. The operative procedure lasted fifty minutes, during which time a transfusion was given. The patient withstood the operation well. During the next two days, however, she became gravely ill, presenting the picture of acute adrenal insufficiency. Her blood pressure remained alarmingly low (on one occasion 75/50), serum sodium concentration fell to a minimum of 127 mEq. per liter and her temperature rose to over 103° without clinical or laboratory evidence of infection. This condition developed in spite of treatment with up to 20 cc. of ACE daily, maintenance doses of DCA, a daily oral and parenteral intake of 10–20 Gm. of sodium chloride, and supportive measures in the form of blood (1000 cc. on the day of operation, 350 cc. on each of the two succeeding days), as well as plasma and glucose infusions, antibiotics and chemotherapy.

The parenteral carbohydrate given the day of operation was inadequate to compensate for the postoperative reduction in food intake and the morning following operation her blood sugar level was 54 mg. per cent. Subsequently hypoglycemia was prevented with frequent parenteral or oral carbohydrate feedings. The one further complication which appeared was a fall in serum potassium content to a minimum of 2.4 mEq. per liter. This was observed on the fourth postoperative day after large quantities of adrenal extract had been administered in combination with considerable amounts of parenteral fluid which, apart from transfusions, contained sodium as the only cation. Although the patient showed no abnormality of neuromuscular function, 1.5 Gm. of potassium chloride was administered by mouth with prompt return of the serum potassium level to normal. It must be pointed out that without repeated determinations of serum potassium concentrations, the administration of potassium might have been hazardous.

The patient's recovery was subsequently uneventful and one year later her condition was satisfactory. However, at the time of writing, information has just been received of her death in an another hospital. Autopsy findings included lobar pneumonia and atrophy of the adrenals.

Case 3, P. H. #813457, H. R. was a 36-year old white housewife in whom the signs and symptoms of Addison's disease appeared coincidently with her first pregnancy. She was initially seen and admitted in the fifth month of gestation at which time she gave a history of six months of increasing pigmentation. Nausea and vomiting dated from the early weeks of pregnancy but persisted and became increasingly severe and were associated with a weight loss of 11 kilograms. There was no history of tuberculosis and her health prior to pregnancy had been good except for chronic sinusitis. On physical examination she was emaciated, appeared chronically and severely ill and there was deep

generalized pigmentation of the skin in addition to brown patches on the buccal mucous membranes. Her blood pressure was 92/60. The level of her serum sodium on admission was 132 mEq. per liter, and her fasting blood sugar 81 mg. per cent. The urinary excretion of 17-ketosteroids determined subsequent to pregnancy was less than 1 mg. in 24 hours. X-ray examination of the chest and of the adrenal area revealed no evidence of tuberculosis. A tuberculin test with 0.1 cc. of O.T. carried to a dilution of 1:100, gave negative results.

Therapy was initially directed at controlling her adrenal insufficiency with the hope that, as she entered the third trimester, the increased blood volume of this period would result in an improvement in her condition. However, this did not prove to be the case, and although her serum sodium values were soon raised to normal levels with DCA and added sodium chloride, her condition continued to degenerate, she developed multiple complications and became progressively debilitated, losing an additional 5 kilograms of

TABLE 1. ADDISON'S DISEASE. CASE 3. PATIENT H.R.: CLINICAL COURSE AND THERAPY DURING PREGNANCY

Month of gestation	Weight, Kg.	Blood pressure, mm. Hg	Serum sodium, mEq./L.	Blood sugar, mg. %	DCA, mg./24 hrs. s.c.	NaCl (Gm./24 hrs.)		Remarks
						Parenterally	Orally	
5	49	88/40	131 138	81	5	2*	3	nausea and vomiting; agranulocytosis; jaundice
6		92/60	138		6	2.1*	4	
7	48	110/68	137	59	6	6.2*	4	
8		128/84	139		6	4.9*	4	
9	43	154/90	137		6	3*	2	

Labor 32 days after expected date of delivery.
* Average daily NaCl supplied by infusion, transfusion or plasma.

weight. In Table 1 her clinical course, laboratory findings and therapy are outlined month by month during pregnancy, and in Table 2, day by day, during and following delivery.

Five major problems arose during pregnancy. The first was the control of her adrenal disease. Nausea and vomiting persisted to term and necessitated frequent infusions of glucose and saline (total of 60 liters) to maintain an adequate intake of sodium and carbohydrate. Secondly, with the hemodilution attendant on the correction of the adrenal insufficiency, a severe anemia and hypoproteinemia became evident (rbc 2.4 million and serum protein 4.5 Gm. per cent). These persisted to term in spite of repeated transfusions (total of 7.5 liters of blood). Thirdly, two months after admission she developed a transient agranulocytosis (wbc 1100, polymorphonuclears 1 per cent) presumably from sulfadiazine which had been administered in small doses prophylactically for a mild right hydronephrosis. After discontinuation of the drug, her white blood cell

TABLE 2. ADDISON'S DISEASE. CASE 3. PATIENT H.R.: CLINICAL COURSE AND THERAPY DURING LABOR AND PUERPERIUM

Day	Temp.	Blood pressure, mm. Hg.	Serum sodium, mEq./L.	Weight, Kg.	DCA, mg. s.c.	ACE*, cc. i.m.	NaCl, Gm.♯	Parenteral fluids		
								Blood cc.	Plasma cc.	Glucose +saline cc.
Day before labor	98.8°	160 / 80		43.0	6		2			
Labor and Caesarean section	99.2°	168 / 98	135			10	18	1000		1000
1 Second operation	100.8°	138 / 80				15	18	1500		500
2	100.4°	142 / 80				15	14	500	500	
3	101.2°	150 / 90	132		5	20	9		500	
4	101.8°	152 / 88	130		5	5	5			500
5	102.4°	154 / 70	135		5		5			
6	103.4°	152 / 92			5		15		500	
7	103.4°	130 / 80			5		10			1000
8	102.6°	130 / 80			5		14			1000
9	102.2°	128 / 84			5		6			
10	100.6°	130 / 78	138		5		6			
Day of discharge		108 / 80	140	41.5	2		7			

* Upjohn's lipo-adrenal cortex.
♯ Sum of parenteral and oral administration.

count gradually returned to normal. Fourthly, shortly after the episode of agranulocytosis she became jaundiced. Homologous serum hepatitis was suspected, but the findings of intermittently clay colored stools, a negative result with the cephalin flocculation test and a bilirubinemia of 5 mg. per cent, were more suggestive of obstruction. The serum phosphatase values rose to a peak of 9.6 Bodansky units, but, in view of the normal elevation in pregnancy (43), this could not be considered as additional evidence in favor of obstruction. The jaundice persisted until delivery, then subsequently cleared. Fifthly, in the month before delivery she showed a moderate hypertension. This appeared to be specifically related to pregnancy since the blood pressure returned to normal following delivery and no elevated readings have been obtained in the subsequent three years of observation.

The patient went into labor thirty-two days after the expected date of delivery. With the onset of labor a slow infusion of saline was started and ACE was substituted for DCA. At the end of ten hours there had been no progress in labor and the cervix remained dilated only one finger breadth. An operative delivery was decided upon and under cyclopropane anesthesia Dr. D. A. D'Esopo performed a classical Caesarian section. The patient withstood the procedure well, maintaining the hypertension throughout. A liter of blood was given during and immediately after the operation. The baby was a 2400 Gm. male infant of 50 cm. length, who died twenty-four hours later from unexplained mediastinal emphysema. The adrenals weighed 11 Gm. (the normal values for infants of this age and size range from 2.8 to 11.0 Gm., according to Benner (44)). There were no other abnormalities.

The day following delivery a second operation was necessitated because of oozing at the operative site and again the patient withstood the procedure well. However her subsequent postpartum course was stormy. As in the case of the previous patient, this woman became acutely ill over a three-day period following delivery, with high fever for which no infectious basis was determined. There was a drop in serum sodium concentration to a minimum of 130 mEq./per liter, although at no time did her blood pressure approach hypotensive levels. In addition to the therapy outlined in Table 2, she was given penicillin and streptomycin. Her temperature gradually returned to normal and her clinical condition improved. The fifth and sixth day after delivery, engorgement of the breasts was noted.

The patient has been observed for three years since delivery. Her general health remains poor but without any residual findings definitely related to pregnancy.

Case 4, P. H. 814847, S. R. was a 24-year old white housewife with known Addison's disease, admitted for vaginal bleeding in the eighth week of her first pregnancy. A diagnosis of adrenal insufficiency had been made eight months previously at the Mt. Sinai hospital, at which time she gave a history of increasing pigmentation and asthenia which had progressed alarmingly following an appendectomy two months before her admission to Mt. Sinai. There was no history or x-ray evidence of tuberculosis. Control of her hypoadrenalism was effected with a daily dose of 1.5 mg. of DCA subcutaneously and 4 Gm. of enteric coated sodium chloride orally.

At the time of her admission to Presbyterian Hospital she showed evidence of generalized pigmentation of the skin with many black freckles and several gray brown spots on the lips, gums, tongue and buccal mucous membranes. Her blood pressure was 100/70. There was a brownish vaginal discharge but the cervix appeared to be closed. Laboratory studies revealed values for serum sodium of 139 mEq. per liter and for fasting blood sugar of 69 mg. per cent. Urinary 17-ketosteroid excretion as determined in the nonpregnant state was less than 1 mg. in 24 hours. X-ray examination of the chest and adrenal area revealed nothing abnormal.

TABLE 3. ADDISON'S DISEASE. CASE 4. PATIENT S.R.: CLINICAL COURSE AND THERAPY DURING PREGNANCY

Month of gestation	Weight, Kg.	Blood pressure, mm. Hg	Serum sodium, mEq./L.	Blood sugar, mg. %	DCA, mg./24 hrs. s.c.	NaCl (Gm./24 hrs.)		ACE, cc. i.m.	Remarks
						Parenterally	Orally		
2		110/70	128 138	76			6		nausea vomiting
3	52.2	95/58	135	55			6		
4		94/68	135	35	376 mg. in pellets implanted 4 mo. before pregnancy		6		
5		86/50	137	63			6		recurrent hypoglycemia
6	54.5	98/56	138	53			4		
7	56.4	100/60	134	67	3	53*	4	30*	
8		98/70	135		3		4		
9	60.3	112/70	134	76	3		4		

Labor 23 days after expected date of delivery.
* Given during a 3-day illness; acute bronchitis.
# Upjohn's lipo-adrenal cortex.

On her third hospital day the patient had a spontaneous complete abortion, with no exacerbation of her adrenal disease.

The patient was discharged and two months later she had 376 mg. of DCA in pellet form implanted at the Mt. Sinai Hospital. Four months after this she again became pregnant. In contrast to Case 3, her condition during pregnancy remained on the whole very satisfactory and she gained a total of 8 kilograms. During this pregnancy she was admitted to the hospital on 4 occasions for observation or for intercurrent infection. She remained in the hospital during the ten weeks prior to delivery. Her course, laboratory findings and therapy month by month during pregnancy are outlined in Table 3, and day by day following delivery, in Table 4.

Control of her adrenal disease during the early months of pregnancy was effected by the implanted DCA pellets and oral administration of 6 Gm. of enteric coated sodium chloride daily. With the nausea and vomiting of the first trimester, she had on one occasion a fall in the level of serum sodium to 128 mEq. per liter, but subsequent determinations were within the normal range. After absorption of the pellets, DCA was given by injection in doses of 3 mg. daily and the sodium chloride was reduced to 3 Gm. per day.

TABLE 4. ADDISON'S DISEASE. CASE 4. PATIENT S.R.: CLINICAL COURSE AND THERAPY DURING LABOR AND PUERPERIUM

Day	Temp.	Blood pressure, mm. Hg	Serum sodium, mEq./L.	Weight, Kg.	DCA, mg. s.c.	ACE*, cc. i.m.	NaCl♯, Gm.	Parenteral fluids	
								Blood cc.	Glucose +saline cc.
Day before labor	99.4°	112/74	135	60.3	4		4		
labor	98.6°	124/80	137		4	10	22	500	2000
1	100.2°	104/70	135			5	10		700
2	99°	102/58			4		5		
3	99.8°	104/70	134		4		5		
4	99°	106/74			4		5		
Day of discharge		108/76	144	54.1	3		5		

* Upjohn's lipo-adrenal cortex.
♯ Sum of parenteral and oral administration.

During the seventh month an intercurrent bronchitis precipitated an Addisonian crisis which responded to penicillin, parenteral fluids and ACE given in addition to maintenance doses of DCA.

Symptoms of hypoglycemia recurred frequently and were documented on many occasions with a decrease in blood sugar levels, at one time to as low as 35 mg. per cent. This problem was fairly well controlled by the use of between-meal and midnight feedings.

The patient went into labor twenty-three days after the expected date of delivery. ACE therapy and a slow infusion of glucose and saline were then begun. Over the course of the next seventeen hours she received a total of 2000 cc. of fluid parentenally, and her condition remained excellent. At the end of this time the patient was delivered under cyclopropane anesthesia, by Dr. D. A. D'Esopo, of a normal female infant weighing 2870 Gm. Following delivery a transfusion of 500 cc. was given. The postpartum course of the patient was entirely uneventful and she was ambulant on the fifth day. The patient's breasts were engorged three days post-partum. No attempt was made at breast feeding and seventeen days post-partum her breasts were soft with only a slight watery discharge.

She has been observed over two ensuing years and her condition remains satisfactory. The baby has been well. At the time of writing, she is again pregnant.

Urinary excretion of hormones

The determinations of urinary excretion of hormonal substances are shown in Table 5. It is evident that the data are limited. The gonadotropic hormones, in the 3 patients in whom these were estimated, were excreted in amounts comparable to those observed in normal pregnancy. The estrogen excretion in M.S. (Case 2) was not abnormal, but in E.G. (Case 1) and S.R. (Case 4) the values were somewhat lower than those usually found

TABLE 5. URINARY EXCRETION OF HORMONES IN 4 PREGNANT WOMEN WITH ADDISON'S DISEASE

Patient	Month of gestation	Gonadotropins, mouse units	Estrone plus estradiol, μg./24 hrs.	Sodium pregnanediol glucuronidate, mg./24 hrs.	17-Ketosteroids, mg./24 hrs	Corticoids, glycogen units
S.R. (Case 4)	2	180,000		8.9		
	5	14,000	326	10.5 1.6	1.8	
	7		89	14.5	1.6 2.7	
	8		320 374		5.7	0
	9		287 442		7.4 3.7	0
	1 year later				less than 1*	
H.R. (Case 3)	7				4.5*	
	8	3,000		70		
	1 year later				less than 1*	
M.S. (Case 2)	4	12,000	94		2	
E.G. (Case 1)	2		30		1.5	

* Determined by the Zimmermann reaction.

in pregnancy, in which levels as high as 750–1000 micrograms have been obtained at term with the fluorometric method used (Jailer, unpublished data). The value for sodium pregnanediol glucuronidate was normal in H.R. (Case 3); the several determinations on S.R. (Case 4) were somewhat low. In the one patient in whom the determination was made, there was no corticoid activity demonstrable in the urine on two occasions. The 17-ketosteroid excretion in S.R. (Case 4) rose during pregnancy to a peak of 7.4 mg., but fell to 3.7 mg. five days before delivery. In H.R. (Case 3) the one determination of 17-ketosteroids made during pregnancy was significantly greater than the determination one year later.

DISCUSSION

It is impossible to draw many conclusions about pregnancy in Addison's disease based on so small a group of patients. However, certain points bear discussion.

Examination of the data on urinary hormone excretion indicates that those substances derived from the placenta, *i.e.* gonadotropins, progesterone (sodium pregnanediol glucuronidate), estrones and estradiol, were excreted in normal or but slightly decreased amount by these patients with hypoadrenalism. From this observation it would appear that a normal maternal adrenal cortex is not necessary for the elaboration by the placenta of sufficient amounts of these hormones to maintain pregnancy.

The absence of corticoid activity in the urine of the one patient (S.R., Case 4) in whom it was assayed, is of interest. Two determinations were made late in pregnancy at which time greatly increased activity is indicated by the values found in normal urine. This normal increase in corticoid excretion late in pregnancy was first thought to reflect excretion from the fetal adrenals, which enlarge strikingly at this time. However, both Venning (1) and Day (45) have found the urine of new born infants to show little or no corticoid excretion. The failure to demonstrate such activity in the urine of pregnant patient S.R. with Addison's disease, offers support to Venning's concept that the increased amounts of corticoid-like material, found normally in pregnancy urine, reflect increased function of the maternal adrenal cortex.

The observed increase in 17-ketosteroid excretion raises a question as to the source of this excretory product in these patients with adrenal insufficiency. Admittedly, in the case of H.R. (Case 3) only one determination was made during pregnancy, and although this value was significantly greater than the one obtained one year later, this could be no more than a reflection of progressive adrenal degeneration. However, in S.R. (Case 4) the several determinations during pregnancy indicate a gradual increase in secretion with a subsequent fall, and Samuels, Evans and McKelvey

(26) have reported an even more striking rise in another such patient. These workers suggested that this rise was due to secretion by the fetal adrenal. However both Talbot and co-workers (46) and Day (45) have reported low 17-ketosteroid excretions in new born infants. Day found values ranging from 0.13 to 3.25 mg. in the 24-hour excretion of 12 such cases, from which it appears unlikely that the fetal adrenal contributes significantly to the increased excretion in pregnancy. Hence, the increased 17-ketosteroid excretion observed in pregnant women with adrenal insufficiency suggests that this excretory product may be derived from some source other than the adrenal. One possible, though purely speculative, interpretation is that the placenta contributes to the observed rise.

Clinical generalizations concerning the management of pregnancy in Addison's disease must be limited, since in only 3 of the patients reported here did gestation continue long enough to permit observations as to its influence upon the underlying adrenal disease. Furthermore the course of these patients was variable—the condition of two, M.S. (Case 2) and H.R. (Case 3) unquestionably deteriorated during pregnancy whereas the general health of the third, S.R. (Case 4), remained excellent. Of the anticipated difficulties, the nausea and vomiting of early pregnancy did not present a serious problem for S.R., although on one occasion a significant fall in the value for serum sodium resulted. On the other hand, in M.S., vomiting necessitated electrolyte replacement with saline infusions and in H.R. it continued to be a most serious hazard throughout pregnancy. Furthermore, H. R. failed to obtain any relief in symptoms, as anticipated, upon entering the third trimester and because of the persistent vomiting, larger amounts of sodium chloride were required to maintain adequate electrolyte balance during gestation than were subsequently needed. However, the daily dosage of DCA was continued unaltered. In S.R. the maintenance therapy with both DCA and sodium chloride did not require alteration as a result of pregnancy.

At the termination of each pregnancy, therapy consisted of the administration of DCA by injection and sodium chloride by mouth; and in addition, parenteral saline, glucose, plasma and whole blood as indicated. Each patient also received hog adrenal lipo-cortex (ACE). This extract has a definite effect on salt and water metabolism and the clinical studies of MacBryde and de la Balze (47), show an effect on carbohydrate. Observations in this hospital have not confirmed an effect on carbohydrate metabolism in humans with doses up to 15 cc. per day (unpublished data); however, its value in Addisonian crises has clinically seemed superior to that of desoxycorticosterone acetate (DCA).

Following delivery, increased manifestations of adrenal insufficiency occurred as predicted in H.R. (Case 3), whereas S.R.'s (Case 4), postpartum

course was unexpectedly benign. Finally it is of interest that lactation occurred in both these women, although its adequacy was not tested by breast feeding.

Of the 2 patients in whom pregnancy was terminated early by operation, one (M.S. Case 2) exhibited a classical Addisonian crisis following hysterectomy. Such a reaction did not occur in E. G. (Case 1) after dilatation and curettage; however, the short episode of shock may have been related to the known vasomotor instability of patients with adrenal insufficiency.

SUMMARY AND CONCLUSIONS

1. Five pregnancies were observed in 4 patients with Addison's disease. All patients survived. One pregnancy ended in a spontaneous abortion, 2 were terminated operatively early in pregnancy, and 2 went to term.

2. In these patients, urinary excretion of gonadotropin was normal. The values obtained for sodium pregnanediol glucuronidate and estrogens, though low, were within the range seen in normal pregnancies. Corticoid activity could not be demonstrated in the urine of 1 patient studied in the third trimester. The urinary excretion of 17-ketosteroids was increased late in pregnancy in the 2 patients in whom gestation proceeded to term.

3. The clinical management of pregnancy complicated by Addison's disease is briefly discussed.

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HORMONAL ALTERATIONS IN MEN EXPOSED TO HEAT AND COLD STRESS

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THE adrenal cortex plays a role in the body's defense against cold (1, 2, 3, 4), but the precise nature of this role has not been clarified. Bilateral destruction of the adrenal gland diminishes resistance to varying types of stress including muscular exercise, burns, infections, shock, trauma, anoxia and cold (2). Histologic studies reveal adrenal hypertrophy (5, 6, 7), and chemical studies show a diminution in the cholesterol and ascorbic acid content of the adrenal cortex in rats exposed to cold (8, 9). In fact, so well established is the relationship between the adrenal cortex and the stimulus of cold, that the latter is used as a standard stress in the biologic assay of adrenal cortical extracts (10).

Indirect evidence that outpouring of the adrenal cortical hormones is responsible for the changes seen in the "general adaptation syndrome" (11) is provided by experimental administration of adrenal cortical extracts (12). There is involution of thymus and lymphoid tissue and fall in circulating lymphocytes following injection of adrenal cortical extracts. The lymphocyte fall has been used as a criterion for adrenal response to stress in human beings (13, 14, 15).

The urinary excretion of 17-ketosteroids has been regarded as an index, to some extent, of the rate of adrenal cortical hormone excretion (16, 17). Some workers found a decreased output with a slow return to normal following trauma, bacterial infections and surgical procedures (16). Others (18, 19) found that in normal individuals, the excretion of 17-ketosteroids usually rose for twelve to forty-eight hours, then fell to a subnormal rate and gradually returned to normal. A sharp rise in 17-ketosteroid excretion immediately after stress with an abrupt fall below normal was also found in another study on 30 patients who had experienced hemorrhage, fracture, burn, infection, or operation (20). Elevated excretion of 17-ketosteroids has been reported in muscular fatigue (21), aircraft pilots (22) and in subjects exposed to cold for two hours (17).

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PHYSIOLOGIC INDEX	CHANGES FOLLOWING 25 mgm. DOSE OF ACTH	CHANGES FOLLOWING REPEATED DOSES
ADRENAL CORTEX (in rats)		
ASCORBIC ACID CONTENT	↓[3]	↑[3]
CHOLESTEROL CONTENT	↓[3]	↑[3]
BLOOD (in human beings)		
EOSINOPHILIC LEUCOCYTES	↓	↓
LYMPHOCYTES	↓	↓ OR O'
HEMOGLOBIN CONCENTRATION AND HEMATOCRIT	O	↓
BLOOD SUGAR		↑ OR O'
SERUM CO_2 COMBINING POWER		↑
SERUM INORGANIC PHOSPHORUS		↓
SERUM URIC ACID	O	O
SERUM PROTEIN AND ELECTROPHORETIC PATTERN	O	O
FREE PLASMA CHOLESTEROL		↓'
URINE (in human beings)		
17-KETOSTEROIDS	O'(↑ AFTER 100 mgm.)'	↑
11-OXYSTEROIDS	↑'	↑'
VOLUME		↓
URIC ACID	↑	↑
CREATININE	O	O OR ↓
URIC ACID—CREATININE RATIO	↑	↑
CREATINE		↓'
SODIUM	↑	↓ OR O'
CHLORIDE	↑	↓ OR O'
POTASSIUM	↑	↑ OR O'
PHOSPHORUS		↑ OR O'
CHOLESTEROL		↓'
TOTAL NITROGEN		↑ OR O
GLUCOSE		↑[2] OR O'
SKIN (in human beings)		
ACNE		↑'

Data from Forsham et al (1948) except where otherwise indicated.
' Reported by Mason et al (1948).
[2] Reported by Conn et al (1948).
[3] Reported by Sayers et al (1945).

FIG. 1. Physiologic changes following administration of anterior pituitary adrenocorticotropic hormone in animals and human beings.

Other workers regard the 17-ketosteroids as an unreliable means of determining adrenal activity (23) since they find variable 17-ketosteroid values in both physiologic and pathologic stresses. It has been shown that the urinary glycogenic corticoids (11-oxysteroids) may be rising while the 17-ketosteroids are falling in a stress situation (24). Until more reliable

methods are developed for assay of the "sugar" hormones (glycogenic corticoids) of the adrenal cortex, the information regarding them will be equivocal in stress studies.

The recent developments in the purification of the anterior pituitary adrenocorticotropic hormone (ACTH) have led to its use in assessing adrenal cortical function. The effects in human beings (25, 26, 27) of ACTH are summarized in Figure 1. It has long been known that the number of circulating eosinophils falls in severe infections and other stress situations (28, 29). In addition, the changes in eosinophils produced by ACTH can be reproduced by injection of Kendall's Compound F (a so-called "sugar" hormone) (26).

The administration of ACTH has been suggested as a means of assessing adrenal cortical reserve (26). A single dose of 25 mg. of ACTH injected intramuscularly produces a fall in circulating eosinophils and a rise in the urinary uric acid-creatinine ratio exceeding 50 per cent in people with normal adrenal cortical reserve. Mild adrenal insufficiency is said to be indicated by a normal fall in eosinophils with only a small rise in the uric acid-creatinine ratio (20 to 50 per cent), whereas individuals with marked adrenal cortical insufficiency show a decrease in eosinophils of less than 20 per cent and an increase in the uric acid-creatinine ratio not exceeding 50 per cent.

Early experiments showed that removal of the thyroid gland interfered with regulation of body temperature (30, 31). Administration of thyroxin to thyroidectomized rats allowed them to survive longer in the cold than untreated thyroidectomized rats (30). It is of interest that iodine storage occurs in the thyroid gland in animals during the summer and that the gland is depleted of iodine in the winter (32). Furthermore low temperatures will produce hyperthyroidism in rats (33). While there are many studies showing a fall in basal metabolic rate on moving to hot climates, (34) there are only a few showing a rise on moving to cold climates (35). The basal metabolic rate of Eskimos is said to be 18 per cent above normal standards (36). An indirect test for increased thyroid activity by measuring the amount of thyroxin necessary to maintain normal gland weight during thiouracil administration showed that more thyroid hormone is secreted in cold than in heat (37).

While the thyroid gland apparently reacts to change in environmental temperature, there is considerable evidence that it plays little part in the initial defense against cold exposure. In the initial days of cold exposure in man, there is a marked fall in basal metabolism followed after the second and third day, by steady rise above control levels (38). In the rat there is evidence of a similar rise in basal metabolic rate (39, 40) but after a longer delay. The thyroid gland of the rat hypertrophies after three weeks' ex-

posure to cold, with a coincident rise in basal metabolic rate. Rats exposed to cold previous to thyroidectomy survive longer in cold after thyroidectomy than thyroidectomized rats not previously exposed (30). This finding, plus the fact that increased thyroid activity occurs only after an initial period of normal or low basal metabolism in human beings and animals, suggest other mechanisms, which apparently adequately defend the organisms during the early periods of cold exposure.

On the basis of the above evidence, an investigation of adrenal and thyroid responses of men subjected to extreme heat and cold was undertaken. The observations were carried out simultaneously with studies of acclimatization to heat and cold. (41).

METHODS

Experimental Design

Three healthy, white males were exposed successively to a preliminary two-week period of physical conditioning; to 19 five and one-quarter hour periods of heat (107°F., dry bulb; 89° F. wet bulb; wind 3 m.p.h.); to 14 five-hour periods of cold (−20°F., wind 3 to 4 m.p.h.); to 5 re-exposures to heat; a five-week interval of no exposure to environmental stress; and finally to 3 re-exposures to heat.

Throughout these periods, studies were made on the circulating eosinophils, absolute numbers of lymphocytes, urinary uric acid-creatinine ratio, urinary 17-ketosteroids, and basal metabolism. In addition, in order to evaluate acclimatization, cardiovascular responses, sweating loss, metabolic rates on treadmills, blood volume, thiocyanate space, water and salt balances, skin and rectal temperatures were measured and are reported elsewhere (41).

Food and fluid intake for each test subject was recorded on a daily basis beginning with the evening meal. No effort was made to control the quantity of food or fluid consumed by the test subjects except that no solid food was allowed after 7:00 p.m., and no fluids after 10:00 p.m. Total 24-hour urine collections were made on each man for every day of the experimental period.

In order to insure basal conditions for metabolic tests the men retired at 11:00 p.m. in their special barracks which were outfitted with the equipment required to conduct these tests. The men were wakened at 7:00 a.m., permitted to void, and then were maintained in a basal state during the next three hours. The urine collected from 7:00 to 10:00 a.m. was used to determine the uric acid-creatinine ratio. In addition, leucocyte counts, differential counts and chamber eosinophil counts were determined on the men daily in the basal state. Basal metabolism was measured at intervals

of several days. On the intervening days, blood volume and "thiocyanate space" were determined.

Experimental Conditions in the Climatic Chambers

Physical conditioning was accomplished by walking the men for two hours on a treadmill at 3.5 m.p.h. for ten days, in a constant temperature room maintained at 68°F. with a relative humidity of 40 to 50 per cent and a wind velocity of 3 m.p.h. During this exercise period the men wore light clothing.

The exposures in the hot room were carried out at 107°F., dry bulb (89°F., wet bulb) with a wind velocity of 3 m.p.h. Each exposure lasted for five and one-quarter hours during which time each man walked on the treadmill at 3.5 m.p.h. for a period of sixty minutes and another of thirty minutes. During the remainder of the time in the chamber, the men sat quietly.

The cold room exposures lasted five hours, during which the men sat in a climatic chamber maintained at −20°F. with a wind velocity of 3 to 4 m.p.h. Each man wore skin and rectal thermocouples, as well as a heavy Arctic uniform. The men walked on the treadmill at 3 m.p.h. whenever the toe or knee temperature fell to 45°F. The duration of walking was for one or more periods of ten minutes until the toe or knee temperature had risen to 50°F. or more. This procedure eliminated the danger of frostbite and at the same time permitted maintenance of the maximal cold stress possible under these experimental conditions.

Methods for Assessing Various Physiologic Changes

Basal metabolic studies were carried out by collecting expired air into 100 liter Tissot spirometers. Duplicate samples were collected and analyzed for oxygen and carbon dioxide by the method of Haldane (42). The uric acid was determined by Archibald's (43) modification of the method of Kern and Stransky (44) and creatinine by the method of Folin as modified by Lambert (45). The 24-hour excretion of the 17-ketosteroids was determined by a modification of the method of Callow *et al.* (46) employing the Zimmermann color reaction and the Wilson and Carter technique for the stabilization of the alcoholic potassium hydroxide (47). A semi-micromethod was established, using diethyl ether extraction in place of the more laborious carbon tetrachloride technique. This method permits the analysis of samples of 50 cubic centimeters or less.

The eosinophil counts were made by the chamber technique, using an eosin-acetone solution and a Fuchs-Rosenthal chamber (28). The absolute lymphocyte count was calculated from the percentage of lymphocytes on a coverslip smear (counting 300 to 400 cells), and the total leucocyte count was determined in the standard fashion.

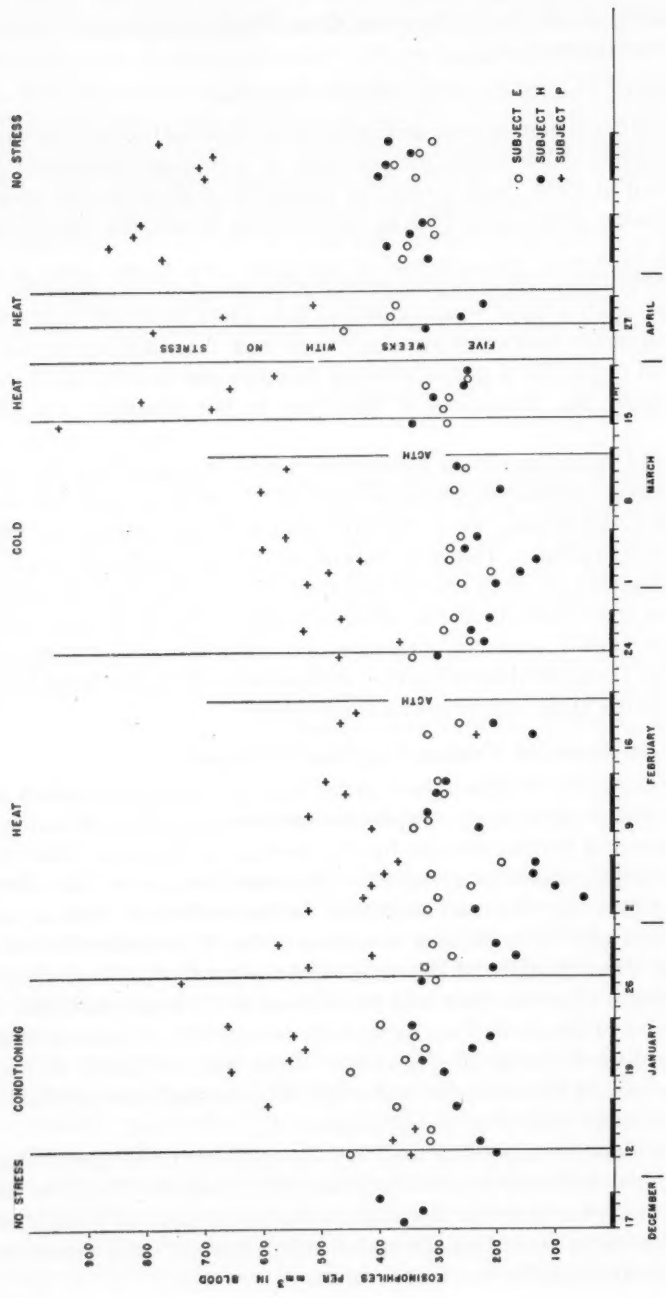

FIG. 2. Counts of circulating eosinophilic leucocytes during heat and cold stress.

TABLE 1. AVERAGE NUMBER OF CIRCULATING EOSINOPHILS PER CUBIC MILLIMETER DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Circulating eosinophils		
		Average	Standard deviation	Number of observations
E	No exercise or exposure	338	26	8
	Conditioning period	363	55	10
	First heat exposure	291	38	16
	Cold exposure	274	33	12
	First re-exposure to heat	286	25	5
	Second re-exposure to heat	407	41	3
H	No exercise or exposure	363	30	8
	Conditioning period	265	54	8
	First heat exposure	214	72	15
	Cold exposure	225	49	12
	First re-exposure to heat	291	45	4
	Second re-exposure to heat	269	41	3
P	No exercise or exposure	769	59	8
	Conditioning period	512	126	9
	First heat exposure	470	109	17
	Cold exposure	511	74	11
	First re-exposure to heat	709	99	5
	Second re-exposure to heat	660	115	3

The anterior pituitary adrenocorticotropic hormone test for the assessment of adrenal cortical reserve was conducted as outlined by Forsham and co-workers (26). The test was conducted under basal conditions and consisted of a two-hour base-line period and a four-hour test period. During the base-line period a two-hour urine sample was collected, as well as blood samples for hematologic studies, blood uric acid (43) and blood glucose (48). Then 30 milligrams of ACTH* were injected intramuscularly. One hour after injection, the test subject voided and this urine sample was discarded, but all urine passed during the subsequent three hours was collected and analyzed for uric acid and creatinine. Four hours after the injection, the hematologic studies, blood uric acid and blood glucose

* This material was kindly furnished to the investigators by Drs. G. W. Thorn and P. Forsham of the Peter Bent Brigham Hospital, Boston, Massachusetts. It was manufactured by the Armour Laboratories, Chicago, Illinois. The preparation used in the studies was lot 32-D, 30 mg. of which had a potency of 25 mg. of the Armour standard extract LA-1-A.

determinations were repeated. The urine collection continued for a full twenty-four hours following injection, for the determination of the 17-ketosteroids. This test was carried out at the conclusion of the series of heat exposures, after the cold exposures, and after a period of no exposure to environmental or exercise stress. Injection of the acid solvent containing no ACTH was administered as an additional control. A 72-hour interval was judged to be sufficient to eliminate any after-effects on subsequent data, since it has been shown that the effects of a single injection of ACTH disappear within twenty-four hours (26).

EXPERIMENTAL RESULTS

It has been reported (41) that these subjects fulfilled the criteria of acclimatization to heat (49) with only slight deacclimatization to the heat after a series of cold exposures. In addition, they showed a tendency toward more rapid vasoconstriction with repeated exposures to the cold.

The results of the daily eosinophil counts are depicted graphically in Figure 2 and the average counts for each man for the various periods are shown in Table 1.

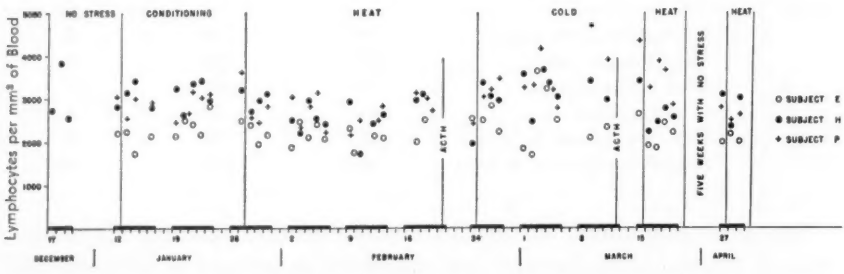

FIG. 3. Counts of circulating lymphocytes during heat and cold stress.

In addition, two of the subjects, H and P, were studied on eight days during which they were subjected to no environmental stress or exercise. These latter values were significantly higher in subjects H and P than those obtained during the initial base-line period (physical conditioning period), which is indicative of the effect of exercise on the circulating eosinophils. Likewise, in general, the number of circulating eosinophils was considerably lower during the various periods of environmental stress, particularly during the initial heat period and the cold period. In subjects H and P the eosinophil values were higher during both periods of re-exposure to heat than during the conditioning period, but lower than the values for the period of no exercise or environmental stress. The other test subject E, had a higher value during the second re-exposure than during

the conditioning period. Under these conditions, the data suggest strongly that circulating eosinophils can be depressed by exercise or a combination of exercise and environmental stress. The degree of depression is presumably related to the severity of the stress imposed.

Simultaneously with the eosinophil determinations, absolute lymphocyte counts were made. Although other workers have reported alterations in absolute lymphocyte counts under stress conditions, no significant differences between experimental periods were observed in any of the test subjects with respect to these values (Fig. 3, Table 2).

TABLE 2. AVERAGE NUMBER OF LYMPHOCYTES PER CUBIC MILLIMETER (ABSOLUTE COUNT) DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Lymphocytes		
		Average	Standard deviation	Number of observations
E	Conditioning period	2,272	299	8
	First heat exposure	2,280	397	15
	Cold exposure	2,508	557	12
	First re-exposure to heat	2,210	345	5
	Second re-exposure to heat	2,055	85	3
H	Conditioning period	3,078	299	9
	First heat exposure	2,703	406	16
	Cold exposure	3,117	506	12
	First re-exposure to heat	2,670	453	5
	Second re-exposure to heat	2,805	341	3
P	Conditioning period	2,872	252	9
	First heat exposure	2,692	407	17
	Cold exposure	3,504	705	12
	First re-exposure to heat	3,620	564	5
	Second re-exposure to heat	2,642	150	3

It will be noted (Fig. 4, Table 3) that there was no significant difference in the daily urinary uric acid-creatinine ratio in any of the test subjects during any of the experimental periods. In connection with these studies, a group of 11 normal males were studied under basal conditions and the average value of 65 determinations on this group was 0.42 with an S. D. of 0.11. This value compares favorably with similar data reported by Forsham *et al.* (26). Analysis of each individual's own mean and variance revealed a range of means from 0.35 to 0.54, and a standard deviation peculiar to the individual.

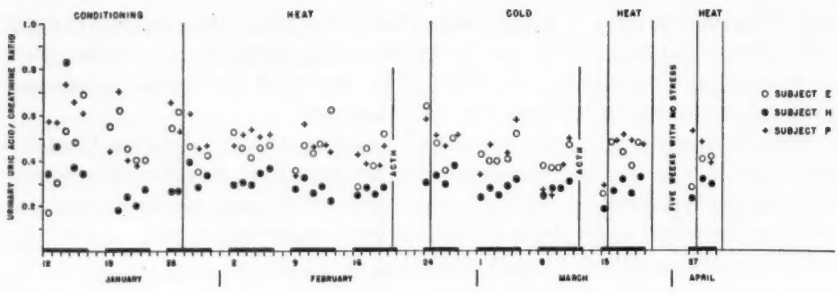

FIG. 4. Urinary uric acid-creatinine ratio during heat and cold stress.

TABLE 3. AVERAGE URINARY URIC ACID-CREATININE RATIOS DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Uric acid-creatinine ratio		
		Average	Standard deviation	Number of observations
E	Conditioning period	0.46	0.15	10
	First heat exposure	0.45	0.08	19
	Cold exposure	0.43	0.08	14
	First re-exposure to heat	0.40	0.09	5
	Second re-exposure to heat	0.36	0.07	3
H	Conditioning period	0.31	0.08	15
	First heat exposure	0.29	0.04	19
	Cold exposure	0.28	0.04	14
	First re-exposure to heat	0.26	0.06	5
	Second re-exposure to heat	0.28	0.04	3
P	Conditioning period	0.52	0.15	10
	First heat exposure	0.47	0.07	19
	Cold exposure	0.42	0.10	14
	First re-exposure to heat	0.44	0.09	5
	Second re-exposure to heat	0.46	0.07	3

The 24-hour excretion of the 17-ketosteroids was determined daily during a period of no exercise or environmental stress, during the last week of the initial heat exposure, during the cold exposure and during the two periods of re-exposure to heat. These data are shown graphically in Figure 5 and summarized in Table 4.

In the period with no exercise or environmental stress, the values are as high as or higher than those observed during the various exposure periods. There was noticeable elevation in the excretion of 17-ketosteroids dur-

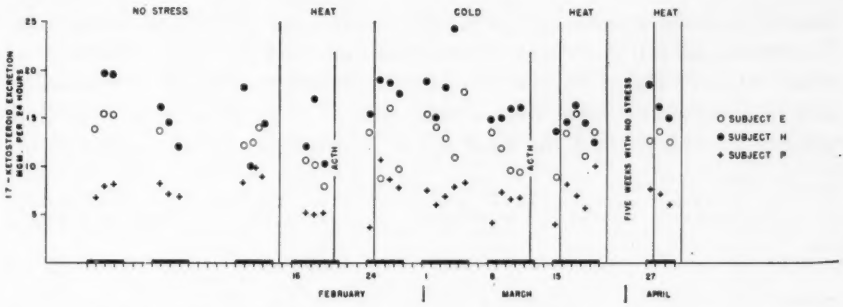

FIG. 5. Urinary 17-ketosteroid excretion during heat and cold stress.

TABLE 4. AVERAGE URINARY EXCRETION OF 17-KETOSTEROIDS (MG. PER 24 HOURS) DURING VARIOUS STRESS STATES

Test subject	Experimental condition	Excretion of 17-ketosteroids/24 hrs.		
		Average	Standard deviation	Number of observations
E	Base-line (no exercise)	13.7	1.2	6
	Acclimatized to heat	9.9	1.3	4
	Cold exposure	12.7	2.8	14
	First re-exposure to heat	12.6	2.6	5
	Second re-exposure to heat	13.0	0.6	3
H	Base-line (no exercise)	16.4	2.9	5
	Acclimatized to heat	12.2	3.5	4
	Cold exposure	17.2	2.7	14
	First re-exposure to heat	14.4	1.5	5
	Second re-exposure to heat	16.6	1.8	3
P	Base-line (no exercise)	7.5	0.9	6
	Acclimatized to heat	5.2	0.1	4
	Cold exposure	7.1	1.8	13
	First re-exposure to heat	7.0	2.4	5
	Second re-exposure to heat	7.0	0.8	3

ing the cold exposure, as well as during the periods of re-exposure to heat when compared to the values during the last week of the initial heat exposure. These differences, however, are not statistically significant.

Results of the administration of ACTH at the conclusion of the initial period of heat exposure, at the end of the cold exposures, and after the period of no environmental stress, together with control data, are summarized in Figure 6 and Table 5. Following the heat exposures, subject P

showed a normal response as shown by the 67.7 per cent fall in eosinophils. The eosinophil fall in subject E was abnormal (29.9 per cent), whereas the other subject's fall of 45.5 per cent in circulating eosinophils is borderline and may represent a low normal response. It is interesting to note that subject E, who showed the least fall in eosinophils, had the greatest in-

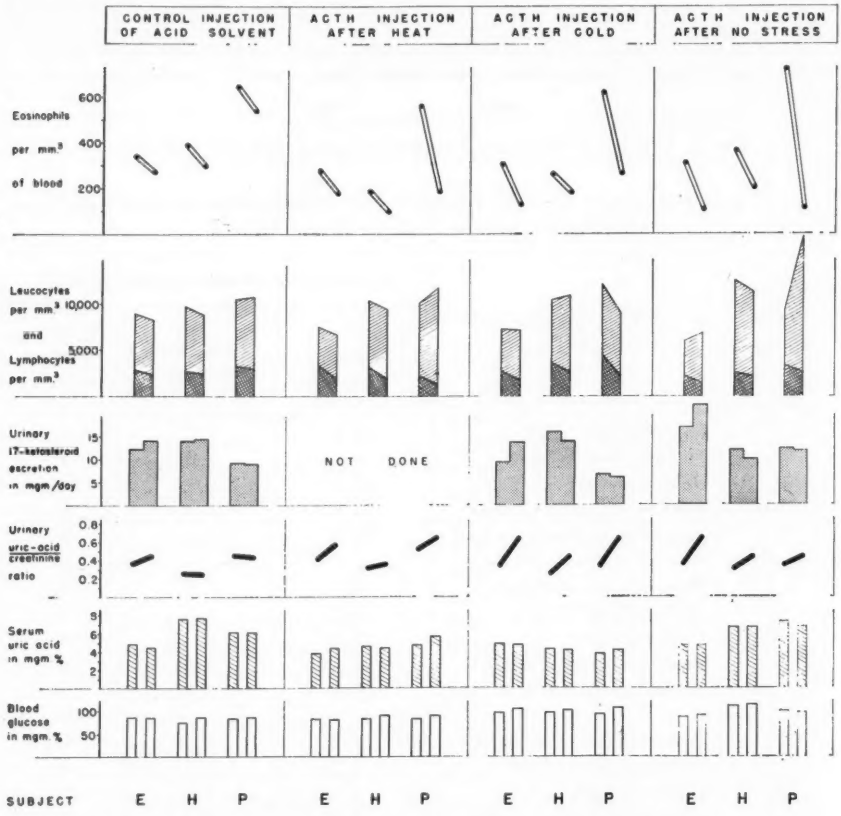

FIG. 6. Results of administration of 30 mg. of anterior pituitary adrenocorticotropic hormone (ACTH) following heat and cold stress.

The first of each pair of results recorded for each test is that found just before injection of ACTH; the second value is that found four hours after injection.

crease in the urinary uric acid-creatinine ratio (41.5 per cent). The other two subjects showed increases of only 13.3 per cent and 15.6 per cent. Following the series of cold exposures, 2 of the 3 test subjects (E and P) had a normal fall in circulating eosinophils with increases in the urinary uric acid-creatinine ratio of 85.7 per cent and 82.9 per cent respectively. In

TABLE 5. RESULTS OF INTRAMUSCULAR ADMINISTRATION OF 30 MG. OF ANTERIOR PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) TO THREE SUBJECTS FOLLOWING VARIOUS TYPES OF ENVIRONMENTAL STRESS

Experimental conditions	Test subject	Test condition	Leucocyte count per cu. mm.	Circulating eosinophils per cu. mm.	Lymphocyte count per cu. mm.	Urinary uric acid/creatinine ratio	Blood uric acid, mg. %	Blood glucose, mg. %	17-ketosteroid excretion, mg./24 hrs.
30 mg. ACTH after 18 heat exposures	E	Pre-ACTH	7,550	257	3,400	0.41	3.8	82.0	not done
		Post-ACTH	6,600	180	1,900	0.58	4.3	81.0	not done
		Per cent change	−12.6%	−29.9%	−44.1%	+41.5%	+13.2%	− 1.2%	
	H	Pre-ACTH	10,400	189	3,100	0.32	4.6	83.0	not done
		Post-ACTH	9,450	103	1,900	0.37	4.4	91.0	not done
		Per cent change	− 9.1%	−45.5%	−38.7%	+15.6%	− 4.3%	+ 9.6%	
	P	Pre-ACTH	10,100	570	2,200	0.52	4.7	82.0	not done
		Post-ACTH	11,850	186	1,300	0.65	5.7	89.0	not done
		Per cent change	+17.3%	−67.4%	−40.9%	+13.3%	+21.3%	+ 8.5%	
30 mg. ACTH after 13 cold exposures	E	Pre-ACTH	7,250	311	2,700	0.35	5.0	98.0	9.45
		Post-ACTH	7,200	133	1,750	0.65	4.8	106.0	13.90
		Per cent change	0	−57.2%	−35.2%	+85.7%	− 4.0%	+ 8.2%	+47.1%
	H	Pre-ACTH	10,500	267	3,700	0.26	4.3	98.0	16.10
		Post-ACTH	11,050	184	2,550	0.45	4.2	101.0	14.00
		Per cent change	+ 6.2%	−31.1%	−31.1%	+73.1%	− 2.3%	+ 3.1%	−9.3%
	P	Pre-ACTH	12,250	623	4,650	0.35	3.8	92.0	6.80
		Post-ACTH	9,000	270	2,150	0.64	4.2	108.0	6.05
		Per cent change	−26.5%	−56.7%	−53.8%	+82.9%	+10.5%	+17.4%	−11.0%
30 mg. ACTH after four weeks of no exercise or extreme exposures	E	Pre-ACTH	6,000	312	2,220	0.36	4.7	87.0	17.00
		Post-ACTH	6,900	106	1,370	0.64	4.7	90.0	22.10
		Per cent change	+15.0%	−66.0%	−38.3%	+77.8%	0.0%	+ 2.7%	+30.0%
	H	Pre-ACTH	12,650	372	2,530	0.31	6.7	110.0	12.10
		Post-ACTH	11,400	206	2,165	0.44	6.7	112.0	10.00
		Per cent change	− 9.9%	−44.6%	−14.4%	+41.9%	0.0	+ 1.8%	−15.7%
	P	Pre-ACTH	9,500	782	3,325	0.35	7.2	99.0	12.10
		Post-ACTH	18,400	105	2,570	0.44	6.6	96.0	11.80
		Per cent change	+94.0%	−86.6%	−22.4%	+25.7%	− 8.3%	− 3.3%	−2.5%
Control acid injection—after no stress	E	Pre-Test	9,000	341	2,870	0.37	4.0	86.0	12.3
		Post-Test	8,250	272	2,310	0.44	4.4	85.0	14.2
		Per cent change	− 8.3%	−20.2%	−19.5%	+18.9%	− 8.3%	− 1.2%	+15.5%
	H	Pre-Test	9,700	388	2,620	0.26	7.6	74.0	14.1
		Post-Test	8,800	300	2,460	0.24	7.7	86.0	14.4
		Per cent change	− 9.3%	−22.7%	− 6.1%	− 7.7%	+ 1.3%	+16.2%	+2.1%
	P	Pre-Test	10,500	654	3,250	0.46	6.2	83.0	9.2
		Post-Test	10,750	544	2,900	0.43	6.2	85.0	9.0
		Per cent change	+ 2.4%	−16.8%	−10.8%	− 6.5%	0.0%	+ 2.4%	−2.2%

subject H, the fall in eosinophils was only 31.6 per cent with a uric acid-creatinine ratio increase of 73.1 per cent. After a period of no exercise or exposure to extreme temperatures, subjects E and P again showed a normal decrease in circulating eosinophils (66 per cent and 86.6 per cent respectively), whereas subject H had a borderline normal response of 44.6 per cent. The increases in urinary uric acid-creatinine ratio were normal for subjects E and H (78.8 per cent and 41.9 per cent respectively) whereas subject P showed an increase of only 25.7 per cent. Injection of the acid diluting medium, with no ACTH, gave negative responses for the various indices. These appraisals of the results are based on the "normal" values published for this test by Forsham *et al.* (26). From this investigation it is not possible to conclude that "borderline" normal responses encountered after stress in these test subjects are truly indicative of a decrease in adrenal cortical reserve.

The basal metabolism of the 3 subjects throughout the various experimental periods is shown graphically in Figure 7. No significant differences were noted in any of the test subjects during the various experimental periods.

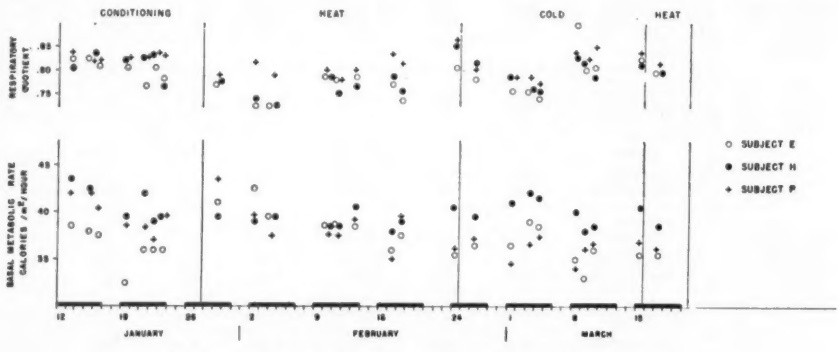

FIG. 7. Basal metabolism during heat and cold stress.

DISCUSSION

This investigation was concerned with the long-term effects of heat and cold stress. It is for this reason that stress was assessed by measurements made on the mornings following the exposures or on a twenty-four hour basis, rather than by short-term measurements of the acute effects of exposure.

Daily chamber counts of the circulating eosinophils yielded more significant data than any of the other aforementioned tests, which furnished either equivocal or negative results. The persistent decrease in eosinophils observed during these various conditions of stress, both environmental and

exercise, possibly offers indirect evidence of "S" hormone activity since it has been previously reported (26) that compound F (an "S" hormone) decreased circulating eosinophils. This indirect technique may offer a new method of assessing response to chronic stress phenomena.

The excretion of 17-ketosteroids during the various stress states is slightly but not significantly elevated over control values. The observed increases fall within the range of daily variations. These data tend to confirm the observations of those workers (24) who feel that the 17-ketosteroids are not concerned with acute stress phenomena.

The urinary uric acid-creatinine ratio did not represent a good index in these long-term studies of stress because of the large daily variation. The change produced by ACTH suggests that the usefulness of the uric acid-creatinine ratio may be confined to acute stress reactions.

Studies of adrenal cortical reserve utilizing ACTH have been suggestive, but not conclusive of a decreased reserve in individuals chronically exposed to severe environmental stress. Because of the very limited number of experiments reported in this field, precise interpretation of the results is difficult. It is conceivable that the stress, although sufficient to produce adequate acclimatization to heat, was not sufficient to tax the adrenal cortical reserve. On the other hand the failure to obtain consistent results might be due to the inability of the adrenal cortex to respond to this stimulating dose, and larger, or repeated, doses would be required to produce more consistently maximal changes in normal subjects. In the present studies considerable variation was encountered in two most sensitive indices, *i.e.*, circulating eosinophils and urinary uric acid-creatinine ratio. A "normal" change in the former was often accompanied by a minimal change in the latter and vice versa, thus indicating some of the difficulty in the interpretation of results.

It has been reported that there is a decrease in basal metabolic rate in the initial phases of heat acclimatization (38, 50) but the results of the present study fail to support these observations. It is believed that the difference in results is not explained on the basis of the degree of heat stress imposed, because in the present investigation, adequate acclimatization by the accepted standards was obtained in seven to ten exposures. It is possible that the increment of change observed previously is too small to be observed consistently, and therefore these changes were not seen in the men used in this study. There are variations both in the heat and cold periods on a day-to-day basis for all of the test subjects, but there is no consistent trend. Likewise, in the cold, no significant changes were observed and this confirms previous investigations (51), although there are reports of increased basal metabolism in human beings exposed to cooler environments (35), as well as in animals subjected to cold stress. It must be borne in mind

that, under the experimental conditions employed, the degree or duration of cold stress might have been insufficient to produce a significantly elevated basal metabolic rate during the period of cold exposure.

SUMMARY AND CONCLUSIONS

Three healthy males were exposed successively after a preliminary two-week period of physical conditioning to 19 five and one-quarter hour periods of heat (107°F. dry bulb, 89°F. wet bulb); to 14 five-hour periods of cold (−20°F.); to 5 re-exposures to heat; to a five-week interval of no exposure to environmental stress or rigorous exercise; and finally to 3 re-exposures to heat.

Measurements of circulating eosinophils, absolute number of lymphocytes, urinary uric acid-creatinine ratio, 24-hour 17-ketosteroid excretion, and administration of ACTH were used to evaluate adrenal cortical responses. Basal metabolic rates were used as an index of thyroid activity.

Evidence indicative of a decrease in circulating eosinophils during environmental and exercise stress is presented and the relation of this observation to the secretion of the carbohydrate hormone of the adrenal cortex is discussed.

No significant differences in the excretion of the 17-ketosteroids were noted in any of the experimental periods.

Adrenal cortical function with respect to environmental stress was not successfully assessed on the basis of daily urinary uric acid-creatinine ratios or absolute lymphocyte counts, due to marked daily variations of these indices.

The use of adrenocorticotropic hormone for the assessment of adrenal cortical reserve yielded only suggestive evidence of a reduction of adrenal cortical functional reserve after periods of environmental stress. The difficulty of interpreting these observations is discussed.

No significant differences in basal metabolic rates were observed in any of the exposure periods.

Acknowledgment

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CHANGES IN URINARY URIC ACID-CREATININE RATIO AFTER ELECTRICALLY INDUCED CONVULSIONS IN MAN

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RECENT studies have provided evidence indicating that increased secretion of adrenal cortical steroid hormones occurs in patients given electroconvulsive therapy for mental disease (1–7). Changes in the uric acid-creatinine ratio four hours after the injection of adrenocorticotropic hormone (ACTH) has been suggested as a measure of adrenal cortical function (8) (9) and accordingly it was considered desirable to measure this ratio in patients given electroshock therapy.

MATERIAL AND METHODS

Fifteen studies were made on 9 women who were given electroconvulsive therapy for mental disease. The ages ranged between 25 and 73 years and the diagnoses varied (Table 1).

The patients voided an hour and a half before the treatment; this urine sample was discarded and another sample was obtained immediately before shock was administered. Thereafter, samples were collected four hours after the treatment and whenever possible at the end of two hours also. In addition, some of the patients voided at other times during the four hours after shock was given; some of these samples of urine were obtained and the times at which they were voided were recorded. In other instances, however, specimens were lost, since the patients, psychotic to begin with and confused after the treatment, voided without attracting the attention of a nurse. In 7 of the 15 experiments all samples of urine voided during the first four hours after shock were collected at intervals accurately recorded; in these 7 instances calculation of hourly excretion of uric acid and of creatinine was possible. In all 15 studies the uric acid-creatinine ratio was calculated from measurements of the concentrations of these two substances in the samples of urine obtained.

Creatinine was estimated by means of Folin's method (10); uric acid was measured according to the method of Benedict and Franke, modified by Folin (11), after the procedure was adapted to the Coleman spectrophotometer.

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TABLE 1. EFFECT OF ELECTROSHOCK ON URINARY URIC ACID-CREATININE RATIO

Case	Age (yrs.)	Diagnosis	Duration of disease	Ratio	Remarks
1	50	Schizophrenia-paranoid	8 years	0.36	Before first shock.
				0.47	4 hours after first shock.
				0.29	Before second shock.
				0.50	4 hours after second shock.
2	25	Schizophrenia-paranoid	2 years	0.32	Before sixty-second shock.
				0.25	2 hours after sixty-second shock.
				0.45	4 hours after sixty-second shock.
				0.29	Before sixty-fifth shock.
				0.49	4 hours after sixty-fifth shock.
3	57	Schizophrenia-paranoid	25 years	0.48	Before first shock.
				0.28	½ hour after first shock.
				0.63	5 hours after first shock.
				0.57	Before third shock.
				0.43	2 hours after third shock.
				0.56	4 hours after third shock.
4	40	Schizophrenia-paranoid	4 years	0.40	Before first shock.
				0.43	2 hours after first shock.
				0.48	4 hours after first shock.
5	44	Manic-depressive, mixed	9 months	0.56	Before sixth shock.
				0.67	2 hours after sixth shock.
				1.20	4 hours after sixth shock.
6	44	Manic-depressive, manic	3 months	0.47	Before third shock.
				0.40	2 hours after third shock.
				0.71	4 hours after third shock.
7	59	Involutional psychosis, melancholia	4 months	0.49	Before second shock.
				0.33	2 hours after second shock.
				0.64	4 hours after second shock.
				0.71	Before third shock.
				0.27	1 hour after third shock.
				0.69	4 hours after third shock.
8	65	Involutional psychosis, melancholia	1 month	0.48	Before first shock.
				0.47	2 hours after first shock.
				0.81	4 hours after first shock.
				0.39	Before second shock.
				0.36	2 hours after second shock.
				1.08	4 hours after second shock.
9	72	Involutional psychosis, melancholia	6 months	0.48	Before second shock.
				0.43	4 hours after second shock.
				0.51	Before fifth shock.
				0.56	2 hours after fifth shock.
				0.51	4 hours after fifth shock.

OBSERVATIONS

Uric Acid-Creatinine Ratio: Before treatment, the ratio was between 0.29 and 0.71, and averaged 0.45 (Table 1). The standard deviation was 0.11.

One half to two hours after check, the ratio lay between .25 and .67 and averaged 0.40; the standard deviation was 0.12. In 8 instances decreases

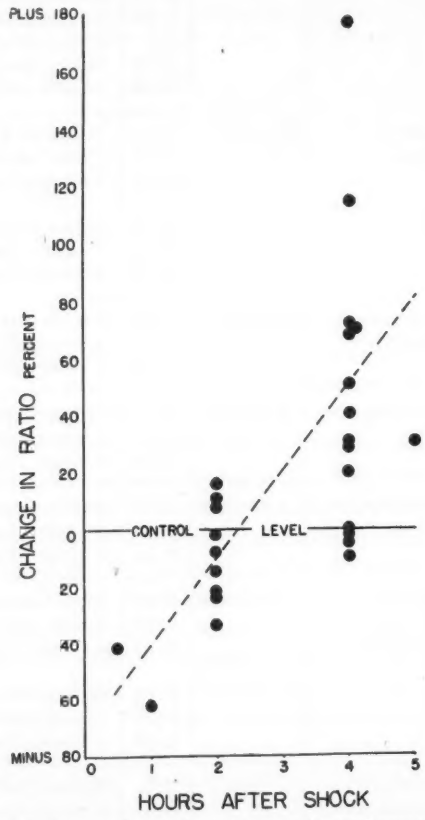

FIG. 1. Relation between change in urinary uric acid-creatinine ratio and time of study after electroshock.

of between 0.01 and 0.34, averaging 0.13, occurred. In 3 instances increases in the ratio of 0.05 to 0.11, averaging .06, were found. The average change in the 11 studies in which urine samples were obtained between one half and two hours after electrically induced seizures, was minus 0.08.

Four or five hours after administration of electroshock, the uric acid-

creatinine ratio was between 0.43 and 1.20, and averaged 0.64; the standard deviation was 0.22. Increases in ratio of 0.08 to 0.71, averaging 0.27 occurred in 11 instances. No change occurred in 1 and decreases of 0.01 to 0.05 occurred in 3. The average change in all 15 experiments was plus 0.19 or 42 per cent of the control average value.

No clean-cut relation between time of voiding of urine after shocks and percentage change in ratio was found although the data indicate the more common occurrence of large changes when the samples were collected later in the period of study (Fig. 1).

TABLE 2. URINARY EXCRETION OF URIC ACID AND OF CREATININE AFTER ELECTROSHOCK

Case	Uric acid excretion					Creatinine excretion				
	mg. per hour			per cent change		mg. per hour			per cent change	
	Before shock	Up to 2 hours after shock	2 to 4 hours after shock	Up to 2 hours after shock	2 to 4 hours after shock	Before shock	Up to 2 hours after shock	2 to 4 hours after shock	Up to 2 hours after shock	2 to 4 hours after shock
2	10	13	19	+ 30	+ 90	28	52	41	+ 86	+ 46
5	14	43	31	+207	+122	27	64	26	+137	− 4
6	8	17	33	+112	+313	16	42	47	+163	+194
7	7	16	16	+129	+129	13	44	24	+238	+ 85
	8	9	34	+ 13	+326	12	36	50	+200	+316
8	13	17	34	+ 31	+162	35	46	32	+ 36	− 6
9	10	11	18	+ 10	+ 80	19	19	35	0	+ 84

Excretion of Uric Acid and of Creatinine: Calculation of the hourly excretions of uric acid and creatinine was possible in 7 experiments. Hourly excretions were markedly variable.

In urine samples collected at one or two hours after shock, the uric acid excretion per hour was between 13 and 207 per cent above the control value; the average was 76 per cent above the control (Table 2; Fig. 2). At the same time the hourly excretion of creatinine was between 0 and 238 per cent above the control value, the average increase being 123 per cent (Table 2; Fig. 2).

In the urine samples collected four hours after shock the uric acid excretion per hour was between 24 and 326 per cent above the control value (Table 2). The average increase above the control values was 161 per cent. At the same time the hourly excretion of creatinine was decreased by 4 and 6 per cent in 2 instances and increased by 46 to 316 per cent in 5; the average change from the control values was an increase of 102 per cent (Table 2).

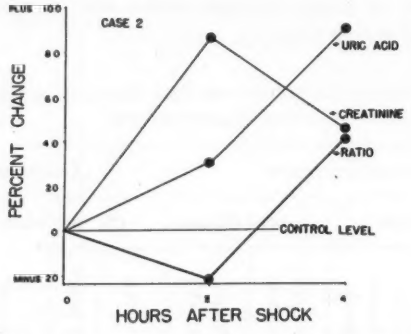

FIG. 2. Uric acid and creatinine excretions per hour after electroshock.

DISCUSSION

The studies of Thorn and of Forsham their co-workers (8) (9) have shown that the urinary uric acid-creatinine ratio increases four hours after stimulation of the adrenal cortex by pituitary ACTH. The utilization of changes in the urinary uric acid-creatinine ratio after injection of ACTH was suggested by Thorn and his co-workers as a device whereby increased output of uric acid might be detected without the necessity for collecting every specimen of urine. When adrenocorticotropic hormone is injected the ratio becomes elevated because the excretion of uric acid four hours later is increased greatly, while the output of creatinine remains unchanged or diminishes slightly. After electrically induced convulsions, however, the output of both uric acid and creatinine is increased markedly; the output of creatinine may show its greatest increase in the first two hours after administration of electroshock whereas as a rule that of uric acid exhibits its most marked rise in the second two hours. Since the two vary independently after electroshock, it is apparent that the interpretation of changes in the ratio is not exactly the same as in the case of changes after injection of ACTH

Changes in ratio two hours after electroshock are not statistically significant although in most instances a decrease occurs. Four hours after the shock, however, the average rise in ratio amounts to almost twice the

standard deviation of the control average. In instances in which the ratio falls initially, the later rise in ratio may be so small in some cases that it does not go much above the control level four hours after shock, *i.e.*, when the effects of adrenal cortical stimulation are believed to be maximal.

The variations in ratio which occur are probably consequent, at least in part, to changes in adrenal cortical function, for other evidences of increased secretion of 11-17-oxysteroids are available; these evidences consist in decreases in the number of circulating lymphocytes (1) (6) and eosinophilic cells (5). On the other hand, it is apparent that other influences also operate to modify the urinary uric acid-creatinine ratio by increasing creatinine excretion after electrically induced convulsions. Studies of blood uric acid levels may be helpful in evaluating more precisely the significance, in regard to adrenal cortical function, of the changes in uric acid output after electroshock.

The pattern of changes found did not appear to be influenced by the patients' ages or the nature or duration of their illnesses.

SUMMARY AND CONCLUSIONS

The urinary excretion of uric acid is increased after electrically induced convulsions in man; this change is usually maximal approximately four hours after administration of electroshock and thereby resembles findings in man after the injection of ACTH. Excretion of creatinine also increases, but this is often most marked during the first two hours after shock. The urinary uric acid-creatinine ratio, therefore, remains unchanged or may fall at first, after which it rises to a level usually well above the control; in some instances, however, the late rise is not very large. The conditions of the present study are so different from those encountered by Thorn, Forsham and associates (8, 9) when they proposed that changes in the ratio four hours after injection of ACTH be used as a test of adrenal cortical function, that precise interpretation of our findings is difficult. Nevertheless, the magnitude of the changes in ratio four hours after electroshock, together with other evidence cited here, indicate increased production of 11-oxysteroids after electrically induced convulsions in man.

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LOSS OF AXILLARY AND PUBIC HAIR IN A PATIENT WITH ADDISON'S DISEASE AND REGULAR MENSTRUATION

A CASE REPORT

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ALBRIGHT and co-workers (1) in 1942 reported the case of a woman suffering from Addison's disease in whom axillary and pubic hair had not developed, in spite of the fact that she menstruated regularly from the age of 16 years. This observation led them to suppose that the sex characteristics in question are not dependent, in the woman, upon the action of estrogens but upon that of some hormone of the adrenal cortex. This belief was further supported by the following facts: It is known that the administration of estrogens does not result in the development of the aforementioned sex characteristics in patients with panhypopituitarism, in which condition there is an insufficiency of the adrenal cortex; whereas estrogens have a very favorable effect in primary ovarian insufficiency, a condition in which the adrenal cortex functions more or less normally. In thyroid insufficiency in women, scarcity of axillary and pubic hair is to be seen, attributed by Albright and associates to the hypofunctioning adrenal cortex observed in this condition, as shown by the decrease in the excretion of 17-ketosteroids. Other writers have published observations which show the importance of the adrenal factor in the development of these sex characteristics. Kepler and co-workers (2) reported a case of a woman with Addison's disease who had very scanty axillary and pubic hair, in spite of having menstruated regularly from the age of 13 years. Mussio Fournier and Proto (3) have published a similar case report.

We here give the case history of another patient of the same type.

CASE HISTORY

Mrs. V. C., a nullipara, aged 43, had a past history of little interest. Puberty was normal and she menstruated regularly with 28/3 rhythm. Her present condition began eight months previously with nausea, vomiting, weakness and darkening of the skin. Physical examination showed pigmentation of the skin and of the buccal mucosa. Her blood pressure was 90/60. There was no axillary hair and but scanty pubic hair. The urinary excretion of 17-ketosteroids was 5.4 mg. in 24 hours. On the twen-

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tieth day of the menstrual cycle there were 20 U.R. (20 international units) of estrogens in the urine. Other clinical and laboratory data are not pertinent to this presentation.

DISCUSSION

This patient with Addison's disease, showing disappearance of axillary and decrease of pubic hair, in whom the estrogenic activity of the ovary was normal, provides additional evidence in favor of the part played by an adrenal cortical hormone in the development of these secondary sex characteristics in women. Albright and co-workers believe that it is testosterone or some similar hormone of adrenal origin. According to these authors, the favorable effect which estrogen therapy has on the growth of axillary and pubic hair in the syndrome of "ovarian agenesis" is due to a stimulating effect upon the adrenal cortex, obtained indirectly through the hypophysis. In spite of the decided influence of the adrenal cortex on the growth of the axillary and pubic hair in women, we believe that further research is necessary before we can discard the possibility of the estrogens acting directly and synergistically in combination with the cortical hormone.

With our associate Albrieux, we are at present studying the effect of applications on the skin of the axillae, of ointments containing either estrogens, testosterone, or both.

SUMMARY

This case report deals with a woman, aged 43, suffering from Addison's disease, in whom axillary hair had disappeared and pubic hair was very scanty, in spite of the fact that she menstruated and had a normal content of estrogens in her urine. The part played by the adrenal cortex and estrogens in the development of these sexual characteristics is discussed.

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Endocrine Review

THE METABOLISM OF THE ESTROGENS: A REVIEW

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THE isolation of the estrogens by Doisy *et al.* (1) and Butenandt (2) aroused considerable interest in their metabolism (origin, action and degradation). Such information is necessary as a guide for their more rational use in therapy, as well as for more academic reasons. As far as the female genital tract is concerned, the estrogens are the most potent known stimulators of growth and cellular division. For example, by means of the colchicine technique, Allen, Smith and Gardner (3) have demonstrated as many as 1585 mitotic figures in one cross section of epithelium of the mouse vagina thirty-seven hours after the administration of but a few micrograms of estrone. It has also been shown that estrogenic stimulation, either endogenous or exogenous, is necessary for the development of certain genital tumors in strains of genetically susceptible animals (Lacassagne (4), Lipshütz (5)). There has been presented some circumstantial evidence that has implicated the estrogens as possible etiologic agents in uterine and mammary carcinoma in the human as well (Corscaden and Gusberg (6), Ayre and Bauld (7), Auchincloss and Haagensen (8)). Consequently a better understanding of the metabolism of these steroids may shed light on some of the very fundamental problems of cellular growth and division as well as neoplastic transformation.

The estrogens have been found in the ovary, placenta, urine and in some species of plants. MacCorquodale, Thayer and Doisy (9) have isolated estradiol from sow's ovary and it is postulated that α-estradiol is the steroid secreted by the ovary. The estrogenic activity of α-estradiol is about 8–10 times that of estrone and 10–15 times that of estriol when assayed by the vaginal smear method on the ovariectomized rat. With other types of assay the comparative values vary somewhat more. (In the human, α-estradiol does not appear to be that much more potent than estrone). The minimum amount of α-estradiol necessary to cause vaginal cornification in the spayed rat is modified by factors such as the strain of rat, the diet, and the housing conditions, but appears to be approximately 0.1 microgram.

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Urinary excretion

The demonstration of estrogens in the urine of pregnant women by Aschheim and Zondek (10) facilitated the isolation and identification of crystalline estrone, estradiol and estriol. The urine of pregnant mares and of stallions is also a rich source of the natural estrogens. Equilin, hippulin and equilenin have been obtained from the urine of the pregnant mare. However, most physiologic investigations have been confined to the human and laboratory animals. It has been demonstrated that although the estrogens are excreted in minute amounts in the normal female, there are two peaks of excretion, one at mid-interval which is considered to bear some relationship to ovulation and the other preceding the menses (D'Amour (11), Werner (12), Gustavson *et al.* (13), Gallagher *et al.* (14), Smith, Smith and Pincus (15), Salter, Humm and Oesterling (16), Jailer (17, 18) and others). There is also a fairly constant excretion of estrogens in the male (Werner (12), Gallagher *et al.* (13)). Due to differences in the technique of bio-assay employed by the various investigators, the exact quantities obtained vary. Slightly higher values have been obtained by some of the recently developed chemical methods (Salter, Humm and Oesterling (16), Jailer (18)). But generally speaking, the equivalent of approximately 5–20 micrograms of estrone as determined by bio-assay are excreted daily by the female between the peaks, at which time the excretion may rise to as high as 60–100 micrograms. The daily urinary excretion in the normal male has been found to vary between 5 and 25 micrograms.

Due to the comparative ease with which these steroids can be extracted from the urine as compared to blood and tissue, the study of urinary excretion has been used as a tool in elucidating certain aspects of estrogen metabolism. Westerfeld and Doisy (19), Fish and Dorfman (20, 21), Heard and Hoffman (22, 23), Pincus and Pearlman (24), Stroud (25), Stimmel (26), Jailer (18) and others have shown that following the injection of varying amounts of estradiol and estrone, a very small percentage of the administered dose can be recovered in the urine. A somewhat similar amount has been found in the feces (Dingemanse and Laqueur (27)). The same phenomenon occurs whether the estrogen is injected into a male or a female. The presence or absence of the end organ (uterus and tubes) or ovaries does not change the percentage of recovery. Previously, Pincus and Zahl (28) had thought the presence of the uterus was necessary for the conversion of estrone to estriol, but subsequent studies have shown this to be untrue (Pearlman and Pincus (29)). Thus, only 2–10 per cent of the administered estrogen can be accounted for and the remainder is either stored or destroyed in the organism. Storage of the administered estrogens by the organism is not an important factor (Zondek (30)), consequently

the great bulk of the estrogens is degraded or converted to substances which have no biologic activity. This mechanism is not easily saturated; for example, Heard and Hoffman (22) have injected as much as 300 mg. of estradiol into a normal male and still recovered about 10 per cent of the amount administered. It has been demonstrated also that estrone can be converted to estradiol, estradiol to estrone and both to estriol. Several investigators have not been able to isolate estriol after the administration of the other steroids mentioned (Heard and Hoffman (23)).

One of the known mechanisms of inactivation is conjugation with glucuronic acid (estriol in man) or sulfuric acid (estrone in the mare and man) (Schachter and Marrian (31), Cohen, Marrian and Odell (32)). The glucoside and ester linkages are broken by acid hydrolysis of the urine prior to extraction with organic solvents. In the urine of normal individuals the nonconjugated fraction is very small (Glass, Edmondson and Soll (33)). It has been claimed that the content of free estrogen is high in pregnant women only during the week preceding delivery (Cohen, Marrian and Watson (34)). The mechanism of conjugation does not account for the great loss of administered estrogen, however, since in most of the experiments cited above acid hydrolysis was performed on the urines.

Smith and Smith (35, 36, 37) have shown that the estrogenic activity of urine can be increased by boiling the urine with hydrochloric acid in the presence of an excess of zinc dust. They have postulated that not only is estrone converted to estradiol (which is about 10 times more active biologically) by this procedure but that some unknown degradation product is reduced back to a biologically active estrogen, "unaccounted-for T_{sn} activity." This has been confirmed by Stimmel (38) who employed a colorimetric method (Kober) for determining the estrogens. Another possible degradation product is Westerfeld's lactone (39) which has been prepared *in vitro* by the oxidation of estrone by hydrogen peroxide. Although the lactone is only 1/7 as active as estrone in stimulating the rat vaginal epithelium, it is supposedly capable of causing the release of the gonadotropic and adrenotropic factors of the pituitary (Smith (40)). Marker and associates (41) claim to have isolated estranediol from urine. It was thought to be a biologically inactive degradation product of estrone, caused by hydrogenation of the "A" ring.

Finally, Fish and Dorfman (21) have isolated β-estradiol from the urine of rabbits which had received α-estradiol by injection. (β-estradiol[1] was originally isolated from mare's urine by Hirshmann and Wintersteiner

[1] Originally, α-estradiol was considered the trans isomer and β-estradiol its cis isomer. However recent work has cast some doubt on the reality of these spatial configurations. Details of this are beyond the scope of this review.

(42)). It has about 1/100 the estrogenic activity of α-estradiol. It has not been found in human urine (Heard and Hoffman (23)).

The difficulty in isolating these metabolic products is great because they occur in but minute amounts, have no estrogenic activity, and consequently cannot be detected by the usual bio-assay. Neither can they be detected by the colorimetric or the fluorometric methods. The former depends upon the presence of a phenolic "A" ring and the fluorescence upon a double bond in the "A" ring (Jailer (18)). Recoveries of urinary estrogens after the administration of exogenous estrogens are of the same order of magnitude by these methods as by bio-assay (Stimmel (26), Jailer (18)). This suggests that one method of degradation must be the destruction of the "A" ring. The solution of this complex problem may occur when it becomes possible to substitute a radioactive carbon directly into one of the rings of the cyclopentanoperhydrophenanthrene nucleus.

The role of the liver in the inactivation of estrogens

Zondek, in 1934 (30, 43), demonstrated the great facility with which the rat can inactivate estrone. It was shown that four hours after the injection of 40,000 m. u. of estrone, he could recover but 5 per cent of the administered dose in the entire animal carcass. The estrogen was practically all transformed before it showed any evidence of having exerted its biologic effect. After hydrolysis, the percentage of recovery was increased somewhat. Heller (44, 45) further demonstrated that liver slices could inactivate estrone and estradiol *in vitro*. The kidney had somewhat the same effect. The spleen, ovary, heart, lung, placenta and endometrium of the rat, on the other hand, enhanced the biologic activity of estrone and its conversion to estradiol was postulated. This exceedingly important point has not been confirmed (Twombly and Taylor (46)). The enzymatic inactivation could be inhibited by heat and sodium cyanide. Singher *et al.* (47) confirmed the *in vitro* inactivation by the rat liver while Twombly and Taylor (46) showed that the transformation mechanism in the human liver was not as efficient as in rat liver. It should be noted that these experiments were carried out under very exact and specific conditions of concentration of tissue and estrogen and there has never been demonstrated any inactivation of large amounts of steroids such as occurs *in vivo*. Liver mince is not as effective as slices in performing this inactivation of α-estradiol, suggesting that the enzyme system involved is dependent upon cellular integrity. It has recently been demonstrated (DeMeio *et al.* (48), Coppedge *et al.* (49, 50)) that nicotinamide and diphosphopyridine nucleotide (cozymase) enhance the activity of liver mince. Israel, Meranze and Johnston (51) added estrone to the perfusate of a liver, heart-lung preparation and obtained inactivation of the estrogen. However, if the liver was excluded from the

circulation, the estrogen could be recovered almost quantitatively. Schiller (52) also performed perfusion experiments and obtained inactivation of estrogens in the perfusate. He demonstrated the conversion of estradiol to estrone to estriol. Stilbestrol is also inactivated by the liver but to a somewhat lesser extent (53).

Oddly enough, certain plant enzymes are capable of inactivating the naturally occurring estrogens (Zondek (54), Graubard and Pincus (55)). The enzymatic activity has been shown to occur along with plant tyrosinase, laccase and phenolase, although not identical with them. This phenomenon also suggests that the destruction of the estrogens may occur in the "A" ring. This enzymatic oxidation is inhibited by cyanide and salicylaldoxime.

Investigations in the intact organism have also demonstrated that the liver is the site of the inactivation process. Talbot (56) fed immature rats carbon tetrachloride by gavage and noticed an increase in uterine weight. Since this did not occur in ovariectomized rats, he postulated that the minute amount of estrogen secreted by the immature rat ovary is usually inactivated by the liver but in the presence of hepatic damage this process fails. Schiller and Pincus (57, 58) demonstrated an increased urinary excretion of administered estrogens, as compared to controls, in rats which were partially hepatectomized. Diet-induced cirrhosis of the liver in rats results in deficiency of the degradation mechanism (Shipley and György (59)). The results obtained with deficiency diets will be discussed subsequently.

Similar results have been obtained in the human. Glass, Edmondson and Soll (60) have shown that not only do male patients with cirrhosis of the liver excrete more estrogens than do normal men but they excrete as much as 83–86 per cent of an administered dosage of estrogen. In infectious hepatitis, the urinary excretion of estrogen is high and shows a fall during convalescence (Gilder and Hoagland (61)). There appears to be a correlation between the excretion of estrogens and hepatic damage, as evidenced by the height of the cephalin flocculation test, in infectious hepatitis. In three male patients with hepatitis the urinary excretion varied from 40–50 micrograms per day (twice normal) when the flocculation test was 4 plus. As convalescence proceeded and the flocculation test reverted to normal the excretion of estrone and estradiol decreased to within normal amounts (Jailer, unpublished data). However, in pregnant women, Zondek and Black (62) could show differences in urinary estrogen excretion only when the hepatitis was extremely severe.

Gynecomastia, vascular spiders, palmar erythema, loss of chest and axillary hair and testicular atrophy have been described in patients with cirrhosis of the liver. These physical signs may be due to excessive estrogen

within the body as a result of decreased inactivation of these steroids (Bean (63), Lloyd and Williams (64)) and decreased androgen production, for which there is some evidence (Glass, Edmondson, and Soll (60), Lloyd and Williams (64)). Gynecomastia was noted in American soldiers who were prisoners of the Japanese. They suffered from malnutrition and vitamin deficiencies. However, although the urinary excretion of 17-ketosteroid was low, estrogen excretion appeared normal (Klatskin, Salter and Humm (65), Salter, Klatskin and Humm (66)). Vascular spiders and palmar erythema do occur during pregnancy when the estrogen content of the blood is exceedingly high.

Cantarow, Paschkis, Rakoff and their collaborators (67, 68, 69, 70) have postulated the existence of a very interesting mechanism in the metabolism of estrogens. They have presented evidence to show the existence of an entero-hepatic circulation similar to that which exists with the bile salts. For example, they have demonstrated higher concentrations of estrogens in human bile in pregnant women at term than in the blood. Estrone has also been isolated from the bile of pregnant cows (Pearlman *et al.* (71, 71a)). Twenty-four to forty-eight hours after the injection of estradiol the bile still contains high concentrations, while none could be found in the portal or hepatic vein blood or in the urine of the dog. Absorption of estradiol from the duodenum of the dog was also demonstrated. After the implantation of a 15 mg. pellet of estradiol into the spleen of a dog, no estrogenic substance could be found in the urine but 178 micrograms could be detected in the bile on the twenty-third day. Thus, it is postulated that the estrogens are not immediately inactivated by the liver but that they are secreted into the bile, flow into the duodenum, and are reabsorbed with the bile salts into the liver. In this fashion there is an entero-hepatic circulation with a gradual degradation of these steroids. Longwell and McKee (72) could not confirm these results and showed a greater amount of estrogen in the urine than in the bile of dogs which had received 1.0 mg. of estrone. The activity in the bile was in the nonketonic fraction (estradiol) while that in the urine was in the ketonic (estrone) fraction. It is postulated that estrone is converted to estradiol in the liver, where it is then degraded.

Interrelation between vitamins and estrogens

Estrus did not occur in rats in which the ovaries had been previously transplanted to the mesentery, a site drained by the hepatic portal system (Golden and Sevringhaus (73)). This has been confirmed in the rabbit (Engel (74)). Similarly, it was demonstrated (G. R. Biskind and Mark (75, 76)) that pellets of crystalline steroids had no estrogenic or androgenic effect when implanted into the spleen of castrated rats. When the spleen was subsequently transplanted to a site drained by the systemic circula-

tion, the estrogen or androgen absorbed from the pellet produced its specific effect on the organism (Biskind and Mark (75)). Thus, when the liver gets the first opportunity to act on the estrogen it can completely inactivate it. However, there appears to be a limit to the capacity of the liver to degrade estrogens. When multiple pellets are implanted into the rat spleen, estrus does occur (Segaloff (77)). Segaloff (78, 79, 80) demonstrated that intrasplenic injections of estrone and estradiol were much less effective in producing estrus as determined by vaginal smear than subcutaneous injection. The differences in effectiveness between the two modes of administration was less marked with estriol. However, Hooker, Drill and Pfeiffer (81) found that in the monkey, pellets of estradiol implanted into the spleen were biologically active, even in the presence of a normal functioning liver. In the rabbit and guinea pig splenic implants of crystalline steroids are inactive, demonstrating hepatic inactivation (Biskind and Meyer (82), Lipschütz *et al.* (83)). Employing the technique of splenic implantation of estrone and estradiol, Biskind and Biskind (84) found that ovariectomized rats would show signs of constant estrus in about ten days when fed a diet deficient in the components of the vitamin B complex. Thus, as a result of dietary deficiency in the components of the B complex, the liver loses its ability to inactivate estrogens. (It still retains its capacity to inactivate androgens, however.) Thiamine and riboflavin are the components necessary for the mechanism of inactivation (Segaloff and Segaloff (85), Singher *et al.* (47)). Singher and his collaborators correlated the efficiency of the degradation mechanism of the liver with its concentration of thiamine and riboflavin. M. S. Biskind (86) elaborated on this mechanism and ascribed many clinical conditions of supposed hyperestrinism, *i.e.* "functional uterine bleeding," cystic mastitis, premenstrual tension, postpartum subinvolution of the uterus, diminished libido and impotence in the male, to a decreased estrogen inactivation as a result of vitamin B deficiency. These conditions are associated with other signs of vitamin B deficiency, for example, glossitis and cheilosis. Biskind further claims that under intensive therapy with the B vitamins these conditions soon disappear. However, his therapeutic claims have not been substantiated, nor do patients with vitamin B deficiency excrete greater amounts of administered estrogen than do normal individuals as one would expect if the inactivation mechanism were impaired (Zondek and Black (63)). Ayre (87) has also claimed that thiamine deficiency and the resultant hyperestrinism occur in women with carcinoma of the cervix uteri, and has postulated an etiologic basis for the carcinoma based on these findings. This has been questioned by Jailer (88) who could find no difference in thiamine saturation between women with uterine carcinoma and selected controls.

Drill and Pfeiffer (89) and Jailer (90), using different methods, have been able to separate the effects of vitamin B deficiency from the concomitant inanition. Evidence was presented to show that in the presence of inanition even huge amounts of the various components of the B vitamins are ineffective in reestablishing the ability of the liver to inactivate estrogens (90). However, it is possible that these divergent results are due to differences in the strain of rats used by different investigators. Some of the animals used by Biskind (Sherman rats) came into constant estrus within a few days after being placed on a vitamin B deficient diet. On the other hand, Sprague-Dawley rats (Drill and Pfeiffer) and Long-Evans rats (Jailer) required eighteen to twenty-one days of a deficiency diet before showing constant estrus smears, although in the latter study the urinary excretion of thiamine fell to practically zero within a few days after the institution of the vitamin B deficient diet.

Another factor in the synthesis or maintenance of the estrogen inactivation mechanism of the liver appears to be the protein content of the diet. György (91) and Unna *et al.* (92) have demonstrated both *in vivo* and *in vitro*, respectively, that estrogen inactivation fails when the animals are on a low protein diet. When methionine and choline were added to the diet of these rats, estrone was again inactivated.

It has been recently shown (Jailer, unpublished data) that if the percentage of protein in the diet is high, estradiol can be inactivated by the rat liver *in vivo* even in the presence of inanition or vitamin B deficiency. Other rats on isocaloric diets containing a lower percentage of protein can not inactivate the estrogen. Thus it would appear that the protein in the diet is the limiting factor in the ability of the rat liver to degrade the estrogens.

A different type of relationship appears to exist between the estrogens and folic acid. Exhaustion of the folic acid content of the body interferes with the normal biologic action of stilbestrol so that it may be possible that in some species at least, the action of stilbestrol is mediated through an enzyme system which requires the presence of folic acid. When chicks are maintained on a folic acid deficient diet and then given stilbestrol, the characteristic increase in the size of the oviduct does not occur (Hertz (93, 94), Kline and Dorfman (95)). The normal response can be attained if folic acid is added to the diet but will not occur on addition of L. casei factor (Hertz). There is also evidence that the same phenomenon may occur in the monkey (Hertz). However, another effect of the administration of stilbestrol in the chick, namely hyperlipemia, is not influenced by folic acid deficiency (Hertz). Koref and Engel (96) have claimed that incubation of estrone with folic acid causes an inactivation of the estrone; however, confirmation of this is lacking.

The possibility of an "activating mechanism"

Not only is the liver concerned with the inactivation of the estrogens but it is possible that it plays a role in some activating process as well. Roberts and Szego (97) have found that in the first twelve to eighteen hours after partial hepatectomy, the uterine response to intravenously administered estradiol is markedly reduced in the previously spayed rat. As time goes on and the liver regenerates, this response becomes essentially normal and at forty-eight to seventy-two hours there is a supernormal peak. From these results, it could be postulated that an hepatic enzyme is necessary for estrogenic activity. However, this would not explain why there is an increased activity of the estrogens when they are applied locally. The phenomenon of the local action of hormones has been recently reviewed by Speert (98). There appears to be some activation mechanism in the animal organism. Emmens (99, 100) has demonstrated that the more common estrogens (estradiol, estrone, stilbestrol, and others) are much more potent when applied locally to the vaginal epithelium than when administered parenterally. However, on the other hand, there is a group of synthetic estrogens (such as triphenylethylene and phenyl stilbestrol) which are much more effective when injected than when applied locally. He termed the latter group "proestrogens," because they must be converted by the animal body to a more biologically active substance. Segaloff (101) has demonstrated that the liver is the site of this conversion, although triphenylchloroethylene is no less effective in a partially hepatectomized rat than in controls. He (102) has recently shown methyl bisdehydrodoisynolic acid (an estrogen originally prepared from estrone by Doisy, in which the "D" ring is opened) to be more active when injected intrasplenically than when given subcutaneously. Eversole, Birnie and Gaunt (103) have demonstrated that although the liver is incapable of inactivating methyl bisdehydrodoisynolic acid when pellets are implanted in the spleen, there was no enhancement of its activity as a result of its passage through the liver.

Effect of other hormones on the metabolism of the estrogens

Although the metabolism of the estrogens has been amply investigated, the influence of other hormones on this metabolism has been barely touched. Pincus and Zahl (26) have shown that when progesterone is administered together with estradiol, there is a greater excretion of the estrogen in the urine. This could mean that progesterone exerts a sparing effect on the catabolism of the estrogens. Smith and Smith (35, 36, 37, 104) demonstrated that the urinary excretion of the apparently nonestrogenic material which may be rendered estrogenic by zinc-hydrochloric acid treatment differs under varying conditions (T_{zn}:T_o ratio). It is least when the partition

of urinary estrogens indicates a maximum rate of estradiol to estrone to estriol conversion and greatest when the total estrogens are low and the rate of conversion is low. The former condition is most pronounced when there is evidence of progesterone secretion or may be attained by the administration of progesterone. This is seen in the toxemias of late pregnancy where the high $T_{sn}:T_o$ ratio can be decreased by progesterone administration. They interpret this as indicating that certain steroids can influence the catabolic mechanism of the estrogens (105). However, Heard, Bauld, and Hoffman (106) could find no difference in the urinary excretion of estrogens in the rabbit, when estradiol was administered alone or together with progesterone. But the ratio of the doses of estrogen to progesterone given by the latter workers was much different from that of Smith and Smith. The ratio of progesterone to estrogen may modify the biologic action of estrogen (Jones and Astwood (107)).

The origin of the estrogens

The steroids are apparently synthesized by the body but little information regarding this mechanism is available. Because of the cyclopentanophenanthrene nucleus common to both cholesterol and the steroids, it has been tacitly assumed that the latter substances are synthesized from cholesterol in the gland concerned. Bloch (108), by use of the isotope technique, has demonstrated the conversion of cholesterol to pregnanediol in the pregnant woman, presumably in the placenta by way of progesterone. Sayers and Sayers (109) have correlated adrenal cortical secretion with a decrease in the cholesterol and ascorbic acid content of the adrenal, under conditions of adrenotropic hormone injection and stress. A somewhat similar phenomenon may occur in the ovary, at least as a result of gonadotropic hormone administration. The injection of pregnant mare serum and chorionic gonadotropic hormone results in a decrease of ovarian "cholesterol" in the rabbit, as shown by histochemical methods (Claesson and Hillarp (110, 111)). This observation has been interpreted as evidence that the decrease in ovarian cholesterol is correlated with the secretion of estrogen. Although Levin and Jailer (112) could show a decrease in total ovarian cholesterol, as determined chemically, in the mature rat ovary soon after the injection of chorionic gonadotropic factor, equine pituitary extract and pregnant mare serum, this decrease in ovarian cholesterol did not occur after the administration of an extract of menopausal urine. The latter gonadotropin is mostly follicle stimulating in action, whereas the former three contain luteinizing properties as well. Consequently, it is entirely possible that the decrease in ovarian cholesterol is indicative of progesterone secretion and not of the production of estrogen. Since the quantity of

estrogen necessary for biologic activity is so minute, it is easy to see why the problem of ascertaining estrogen precursors is so difficult.

Addendum

Since the completion of this manuscript, there have appeared two publications in which the authors have employed radioactive labeled steroids. Twombly, McClintock and Engelman (*Am. J. Obst. & Gynec.* **56:** 260, 1948) have prepared 7,8 dibromestrone from equilin, employing radioactive bromine. Administration of this substance resulted in a localization of the radioactive bromine in the gall bladder and intestinal tract. These observations support the theory of an enterohepatic circulation. Albert, Heard, Leblond and Saffran (*J. Biol. Chem.* **177:** 247, 1949) used iodinated derivatives of estradiol consisting of mono- and diiodo-α-estradiol containing I^{131}. The outstanding feature of these observations was also the accumulation of the labeled material in the gastro-intestinal tract. The next highest concentration of radioactivity was found in the mammary glands. In neither report was there any evidence of localization of the labeled material in the ovary, uterus, vagina or liver. It should be emphasized, however, that the addition of bromine or iodine to the steroids causes a loss of estrogenic activity, so that the substances administered are not true estrogens.

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LETTERS TO THE EDITOR

ETHINYL ESTRADIOL

To the Editor:

IN THE recent excellent review on *Ethinyl Estradiol* by Dr. Kenneth W. Thompson, which appeared in this Journal (**8:** 1088–1098 (Dec.) 1948), a statement was inadvertently made which would seem to indicate that in human subjects the material was equally potent whether given by mouth or by intramuscular injection.

We have been interested in this estrogen for some time and, as mentioned in the review, (Segaloff, A.: The intrasplenic injection of some synthetic estrogens and proestrogens, *Endocrinology* **34:** 335–339 (May) 1944) have reported that, in the rat at least, there is marked hepatic inactivation of ethinyl estradiol.

Because of this, studies of the comparative oral and intramuscular potency of ethinyl estradiol were undertaken in man. A more extensive analysis of the data obtained was presented at the Laurentian Hormone Conference (Segaloff, A.: Metabolism of estrogens with particular emphasis on clinical aspects of physiology and function of ovarian hormones, *Recent Advances in Hormone Research*, Volume 4. In press).

The method of Allen (Allen, W. M.: The biological activity of various estrogens, *South. M. J.* **37:** 270, 1944) was employed for assessment of the estrogen. This method involves the administration of estrogens to women in the menstrual age group who have been ovariectomized but who have intact uteri. The end point for the assay is the occurrence of uterine bleeding after a standard period of administration. Using Allen's method, we have found that our 50 per cent bleeding dose for women following oral administration is 0.35 mg. per week, which agrees well with Allen's 50 per cent bleeding dose of 0.4 mg. per week. In the same patients, further studies were undertaken with the injection method, using ethinyl estradiol dissolved in sesame oil. It was found that 0.08 mg. per week was sufficient to produce withdrawal bleeding in 5 of 10 trials. This, we feel, indicates that there is approximately a fivefold difference between the potency by intramuscular injection and the oral potency of ethinyl estradiol in the human subject. This agrees well with data reported for other estrogens administered by various routes and utilizing various other end points.

This letter is not intended to detract from Dr. Thompson's article in any way but offers additional data which as yet are unavailable in the litera-

ture. We trust this addition will extend the usefulness of Dr. Thompson's very fine review.

From the Department of Medicine, School of Medicine, The Tulane University of Louisiana; and the Endocrine Research Laboratories of the Alton Ochsner Medical Foundation of New Orleans, La.

ALBERT SEGALOFF, M.D.
April 15, 1949.

TO THE EDITOR:

My attention has been called to inaccurate wording of one statement in my recent review of "Ethinyl Estradiol" (*J. Clin. Endocrinol.* **8**: 1088–1098 (Dec.) 1948), as follows: "This estrogen has an estrogenic activity similar to α-estradiol, and its oral potency approaches its injection potency." The fact is that the injection potency of ethinyl estradiol in man has not been reported. I was aware of this, and intended that the oral potency of ethinyl estradiol be compared to the injection potency of α-estradiol and its esters. Yet, alas, I failed to detect the "garbling" of the sentence, and thus offer my apologies to your readers.

Dr. Albert Segaloff has made some observations of the effect of injections of ethinyl estradiol in women, and I think your readers will be pleased to see his data.

KENNETH W. THOMPSON, M.D.
April 19, 1949.

20 Main Street,
Orange, New Jersey.

British American Exchange Fellowships in Cancer Research

AWARDED UPON RECOMMENDATION OF THE COMMITTEE ON GROWTH OF THE NATIONAL RESEARCH COUNCIL

British American Exchange Fellowships in Cancer Research of the American Cancer Society, awarded by the Society upon recommendation of the Committee on Growth of the National Research Council, are offered to citizens of the United States for advanced training and experience in Great Britain in specialized fields of investigation pertaining to the problem of cancer. Similar fellowships are awarded by the British Empire Cancer Campaign to British scientists for study in the United States. The Society and the Committee on Growth welcome the cooperation of universities and hospitals in making known these opportunities.

Fields of Study

These fellowships are awarded by the American Cancer Society to provide specialized training for American investigators in Great Britain where opportunities exist for study in facets of research in malignant disease not widely available in this country. Training for an equal number of young British scientists selected by the Campaign will be provided in this country. The Society, the Committee on Growth, and the British Empire Cancer Campaign believe that the training of young men and women in the many complex disciplines of modern scientific thought and techniques are fundamental to a sound approach to the problem of human cancer.

Candidates will be favored who express intention of following a career in a discipline applicable to the study of growth. In evaluating fellowship applications the Committee will keep in mind potential applicability of the proposed training program to problems of neoplastic growth in specialized scientific areas in which superior facilities exist in Great Britain.

Qualifications of Applicants

Fellowships are open to citizens of the United States who possess the degree of Doctor of Medicine, Doctor of Philosophy, or Doctor of Science. They are intended for young men and women embarking upon a career in clinical medicine or basic research, and also for more mature candidates desiring to extend their fields of competence in these fields. Applicants must be able to demonstrate that they have qualifications necessary to pursue investigation in the fundamental sciences or in clinical medicine.

Applications should state the institution where the fellow plans to work in Great Britain; the individual under whom the fellow desires to work; what problem he intends to investigate; when he wishes to start.

Conditions of Appointment

Fellowships will be awarded for a period of one year.

The annual stipend will be £1000 ($4,020.00). An allowance of $600.00 is made for travel to the site of the fellowship in Great Britain.

University staff appointment, with teaching duties agreeable to the fellow, is permitted, provided it carries no additional salary and provided it is acceptable to the Committee on Growth, the American Cancer Society, and the British Empire Cancer Campaign. No other remunerative work will be permitted during the tenure of the fellowship.

Fellowship appointments are subject to the condition that once accepted, they will not be vacated or the place of work changed within the period of tenure without the consent of the Committee on Growth, the American Cancer Society, and the British Empire Cancer Campaign.

Time of Application

Application forms for British American Exchange Fellowships in Cancer Research may be procured from and submitted at any time to the *Executive Secretary of the Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Ave., Washington 25, D. C.* Favorable action by the Committee on Growth takes the form of a recommendation to the American Cancer Society, and is subject to approval by the Society. Fellowships will be made effective at the convenience of the institution and the fellow.

Location of Work

British American Exchange Fellowships in Cancer Research are not granted to institutions. The place where the applicant chooses to work and the person under whose guidance he wishes to work must be approved by the Committee on Growth and the British Empire Cancer Campaign. Universities or institutions in which fellows plan to work will supply necessary facilities and equipment.

Reports

Within thirty days after the termination of each year of the fellowship, the fellow is required to submit to the Committee on Growth a report of his work during that period.

Publications

It is understood that results of work carried out by a fellow shall be available to the public through the approved scientific channels without restriction. Publications should include a statement that the investigation was carried out during tenure of a British American Exchange Fellowship in Cancer Research of the American Cancer Society and the British Empire Cancer Campaign, recommended by the Committee on Growth of the National Research Council. The Comptroller, American Cancer Society; Executive Secretary of the Committee on Growth; and the General Secretary of the British Empire Cancer Campaign should be notified immediately of acceptance of manuscripts for publication, in order that reprints may be ordered.

The Laurentian Hormone Conference

THE Laurentian Hormone Conference of the A.A.A.S. will hold its 1949 meeting at the Forest Hills Hotel, Franconia, New Hampshire, September 12 through 17. The following program has been arranged:

I. Ovarian Physiology and Function

Biology of the Ovary
Dr. A. S. Parkes, National Institute for Medical Research, London, England
Monday evening, September 12

Physiology of Estrogenic Hormones
Drs. K. E. Paschkis and A. Rakoff, Jefferson Medical College
Tuesday morning, September 13

Maintenance of the Corpus Luteum and Physiologic Actions of Progesterone
Drs. James T. Bradbury, Willis E. Brown and Laman A. Gray, State University of Iowa and University of Louisville
Tuesday morning, September 13

The Vasculature of the Ovary and Ovarian Function
Dr. S. R. M. Reynolds, Carnegie Institute of Washington
Tuesday evening, September 13

II. Chemistry and Physiology of the Sex Hormones

Studies with Estrogen Conjugates
Drs. G. A. Grant and D. Beall, Ayerst, McKenna & Harrison, Ltd.
Wednesday morning, September 14

Chemical Methods for the Estimation of Steroid Hormones and their Metabolites
Dr. Lewis Engel, Massachusetts General Hospital
Wednesday morning, September 14

III. Pituitary Physiology and Function

Pituitary Control of Steroid Secretions
Dr. Roy O. Greep, Harvard School of Dental Medicine
Thursday morning, September 15

The Present Status of Posterior Lobe Hormones
Dr. Robert L. Noble, The University of Western Ontario
Thursday morning, September 15

IV. Humoral Mediators in Nervous Transmission

Sympathetic Neurohumors
Drs. M. L. Tainter and F. P. Luduena, Sterling Winthrop Research Institute
Thursday evening, September 15

The Acetylcholine System in Neural Function
Dr. Ralph W. Gerard, The University of Chicago
Friday morning, September 16

V. Hormones and Tumors

Experimental Endocrine Tumors
Dr. George W. Woolley, Roscoe B. Jackson Memorial Laboratory
Friday morning, September 16

The Virilizing Syndrome in Man
Drs. Louis J. Soffer, J. Lester Gabrilove, Joseph W. Jailer, and Mildred D Jacobs, The Mount Sinai Hospital
Friday evening, September 16

VI. Mechanisms of Hormone Action

Effects of Hyperglycemic Factors of the Pancreas and of Epinephrine on Glycogenolysis
Dr. Earl Sutherland, Washington University, St. Louis
Saturday morning, September 17

Hormone-Enzyme Relationships
Dr. Roland K. Meyer, The University of Wisconsin
Saturday morning, September 17

Attendance at this Conference is limited by the accommodation available at the hotel, but the Committee on Arrangements invites applications for attendance from interested investigators and specialists in the hormone field. The Committee on Arrangements consists of R. W. Bates, E. R. Squibb & Sons; R. D. H. Heard, McGill University; A. D. Odell, Charles E. Frosst & Co.; E. C. Reifenstein, Jr., Sloan-Kettering Institute; A. White, School of Medicine, University of California at Los Angeles; and G. Pincus, Chairman, The Worcester Foundation for Experimental Biology. Applications for attendance at the Conference should be sent to the Chairman at 222 Maple Avenue, Shrewsbury, Massachusetts. Applications to be considered by the Committee must be received by June 6, 1949.